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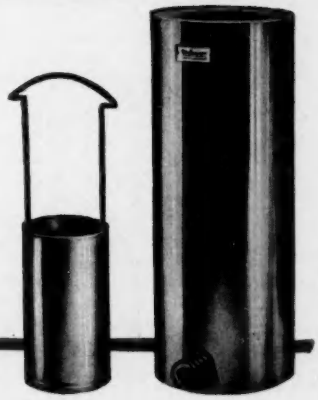

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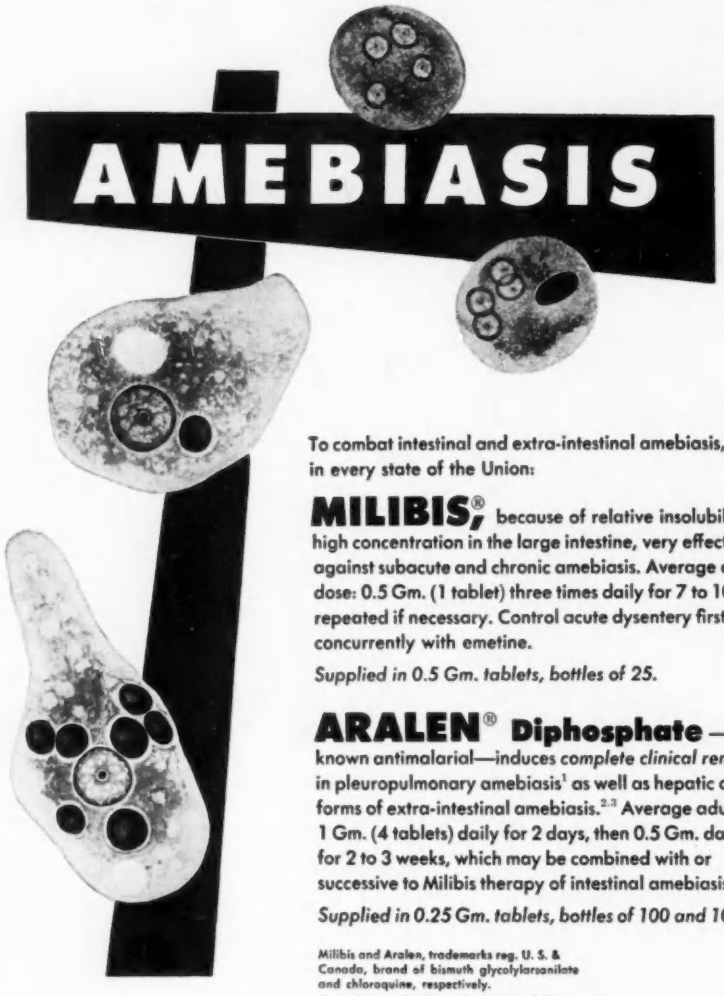

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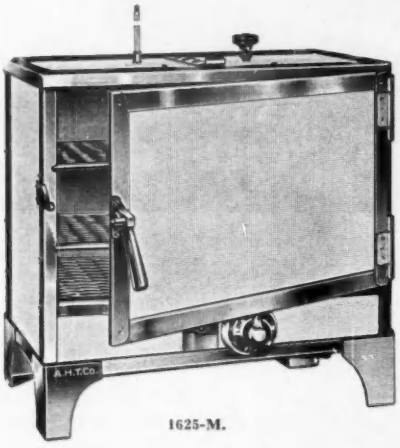

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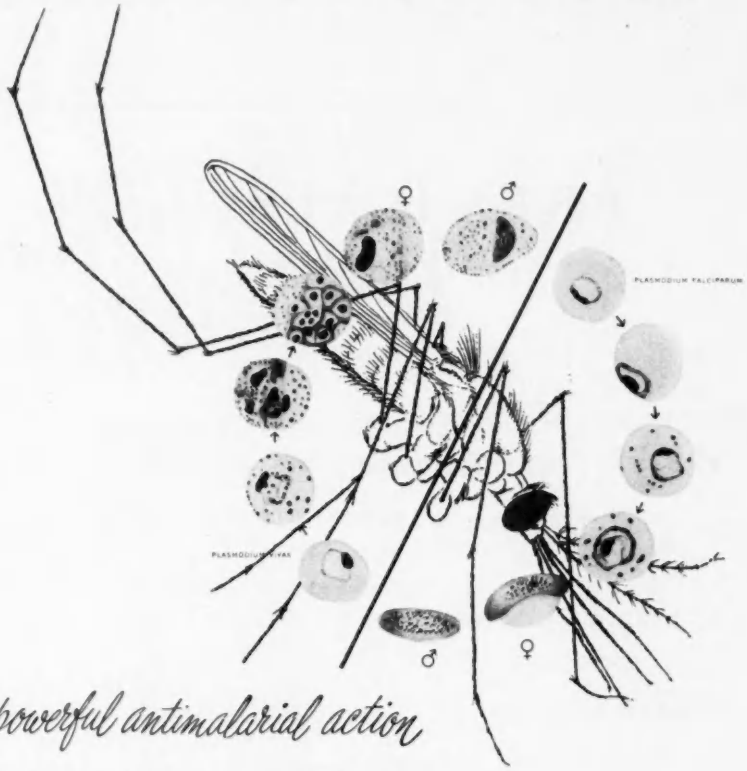

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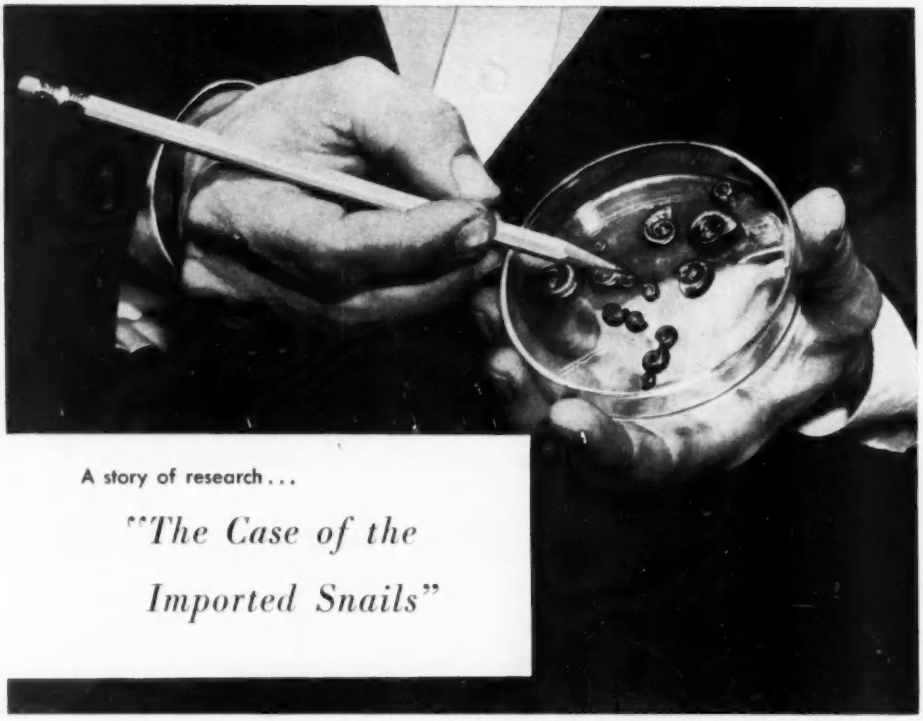

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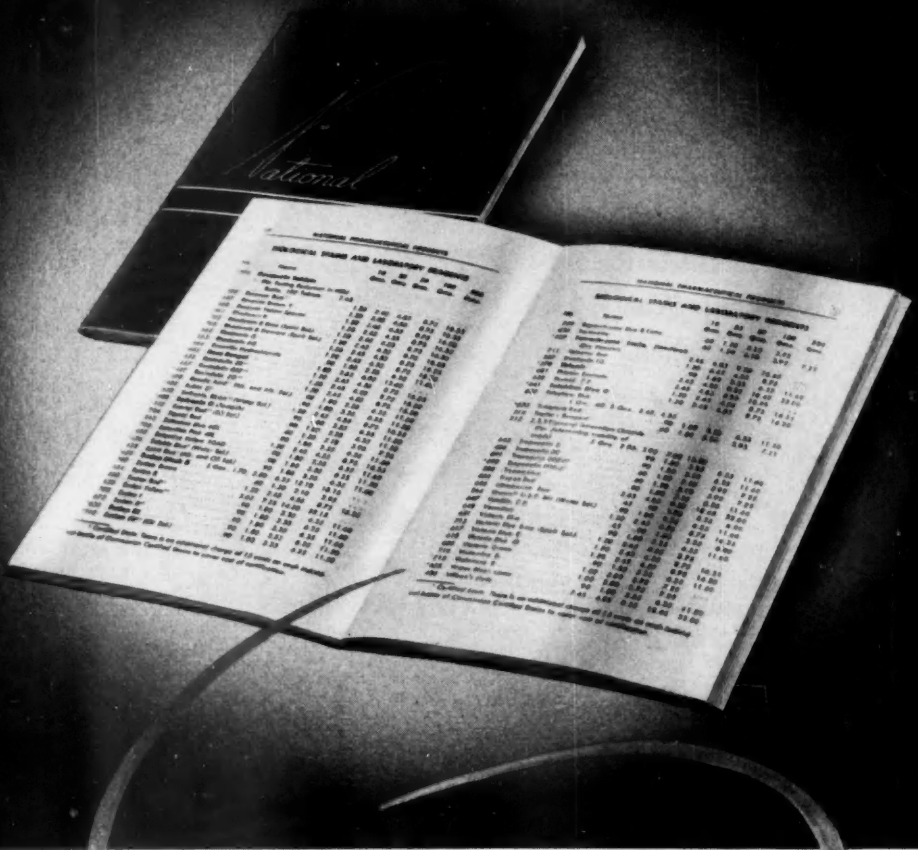

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INTRODUCTION

The reliability of the complement fixation test in amebiasis is still being questioned by many investigators.

Because of relatively high antibody titers in hepatic amebiasis, which may be picked up even with weak antigens, almost all who tried the test in acute amebic involvement of the liver obtained a good percentage of positive reactions. The same agreement is not observed in intestinal amebiasis. While Craig (1933) using human blood, reported 90% accuracy with his test and Bozicevich (1950) obtained 85% of positive complement fixation tests in individuals harboring *Endamoeba histolytica* in the stools, Paulson and Andrews (1938) do not believe the accuracy of their test to be over 47%. Recently, Dolkart *et al.* (1951) found only 31.7% positive complement fixation tests in confirmed intestinal amebiasis, while positive complement fixation tests were observed in 68.3% of cases not harboring *E. histolytica*.

In order to avoid a high percentage of false positive reactions, there is a tendency to dilute the patient's sera, to reduce the amount of antigen per tube or shorten the time of fixation. When one of these factors is introduced the percentage of false positive tests is somewhat reduced. However, the sensitivity of the test suffers to such a degree that with weak antigens and low antibody titers the false negative tests are extremely frequent and render the complement fixation test completely impractical in intestinal amebiasis. Hussey and Brown (1950) who adapted their test to hepatic amebiasis by reducing the time of fixation to four hours, observed 83.4% positive reactions in hepatic amebiasis, with only 2.4% positive complement fixation in intestinal amebiasis.

The clue to a reliable complement fixation test in amebiasis obviously lies in a good antigen. An antigen with no tendency to render the test anticomplementary, would permit picking up cases with low antibodies by allowing the use of increased amounts of antigen and a long fixation period. Its specificity would exclude false positive reactions despite the use of undiluted sera.

The purpose of the paper is to present the Micro-Kolmer technique used in this laboratory, a modification of the Kolmer-Wassermann technique, with emphasis on a simple method of producing a fairly good antigen.

MATERIALS AND METHOD

Antigen

The antigen was produced by modification of the original method of Rees, Bozicevich, Reardon and Jones (1942) using a modified Boeck and Drbohlav

* We are indebted to the staff members of the Medical Service of this hospital for the clinical information made available to us in this study.

medium (1925). The medium was prepared with aseptic precautions as follows:

1. Very fresh whole eggs were emulsified by shaking with glass beads and then filtered through gauze.

2. Locke's solution consisted of:

Sodium chloride	8.0 gm.
Calcium chloride	0.01 gm.
Potassium chloride	0.2 gm.
Magnesium chloride	0.01 gm.
Disodium phosphate	2.0 gm.
Sodium bicarbonate	0.4 gm.
Monosodium phosphate	0.3 gm.
Distilled water	1,000 ml.

The salts were added in the order described to about 500 ml. of distilled water and the solution was then made up to 1,000 ml. The pH was adjusted to 7.1 and the solution filtered through a Seitz filter. The pH was readjusted if necessary. This solution served to dilute the filtered eggs in the proportion of 27 ml. for each 100 ml. of egg emulsion, and as an overlay for the finished medium.

The egg-Locke solution was distributed in 6 ml. amounts in 150/20 mm. screw cap tubes and coagulated in the inspissator at 80°C., at which temperature it was kept for two hours. The tubes were stored in a refrigerator.

To the egg tube before use, 9 ml. of Locke solution and a loopful of rice flour (about 15 mg.) were added aseptically. The rice flour[1] was previously sterilized, by placing small amounts in cotton-plugged test tubes, in a dry oven at 150°C. for two hours. It was added with the aid of a flamed platinum loop twisted so as to form a spoon-like surface.

3. The culture of *E. histolytica* strain 103 growing in symbiosis with a single species "t" organism was used.[2]

The inoculum was obtained from cultures incubated at 37°C. for 48–72 hours. Contaminated cultures were discarded. Cultures containing amebae were incubated an additional 48 hours. After pipetting off the upper 5 ml., the remaining culture overlay was mixed using a 5 ml. pipette, provided with a rubber bulb, the tip of the pipette almost touching the bottom of the tube. The fluid was transferred to a large Erlenmeyer flask and stored in a deep-freeze at −18°C. For a period of four months, overlay from successive subcultures was added to the same flask. This type of collection was necessary to obtain a pool containing *E. histolytica* of different sizes and stages. The culture tubes from which the pool was made contained from 62,000 to 830,000 amebae. The number of amebae in each culture tube was not necessarily higher when the inoculum contained more amebae. The fact that the number of amebae in culture tubes may be the same regardless of the size of the inoculum was previously observed by Brackett and Bliznick (1947).

[1] Rice flour obtained from Quong Yuen Shing & Co., 32 Mott St., New York 13, N. Y.

[2] Culture originally obtained from Dr. H. W. Brown, Columbia University, New York. Later a strain of the same origin was obtained from the laboratories of the Department of Health, City of New York.

When a sufficient amount of culture was thus collected, the amebae were disintegrated by repeated freezing and thawing. A 10% solution of Merthiolate was added to the measured culture lysate to make a concentration of 1:1,000. The turbid solution was centrifuged at 4°C. at 3,500 r.p.m. for 1 hour. Then the supernatant was centrifuged twice more at 0°C. at 18,000 r.p.m. (45° angle, radius 7 cm., 25,000 X G.) for 30 minutes. The opalescent fluid was transferred into Visking tubings and dialized for 24 hours in running water. Dialysis was repeated three times for the same period of time in distilled water, in a refrigerator. The isotonicity was restored by finally placing the dialysing tubes overnight in Kolmer saline. A 10% Merthiolate solution was added in order to reestablish a concentration of 1:1,000. The antigen was transferred to screw cap tubes and stored at −18°C.

Patient's serum

The serum must not be chylous. Aseptic technique was not practiced, but one drop of 10% Merthiolate solution for 1 ml. of serum was added for preservation and serum stored either in the deep freeze (−18°C.) or in a refrigerator (4°C). Serum was inactivated 30 minutes at 56°C. prior to the test. Only the amount required for retesting with different lots of antigen was reinactivated at 56°C. for 10 minutes.

Kolmer saline

0.85 gm. % of NaCl, to which 10 mg. % $MgSO_4$ was added.

Complement

Lyophilized complement was reconstituted as directed. It was diluted 1:30 for hemolysin titration and 1:40 for complement titration.

Hemolysin

Lots of hemolysin prepared by the laboratories of the Veterans Administration Hospital, Hines, Ill., or by the laboratories of the Department of Health, City of New York, were used.

Hemolysin titration

The hemolysin titer was determined each time the complement fixation test for amebiasis was performed, as described in the Kolmer-Wassermann technique for syphilis (Kolmer *et al.* 1941). It was arranged that the Micro-Kolmer test for amebiasis be done on the same day as the Kolmer-Wassermann test for syphilis, using the same hemolysin solution, sheep cells and complement. In the Kolmer-Wassermann method for syphilis the final hemolysin solution contains 2 units per 0.5 ml., and since 0.05 ml. of hemolysin solution are used per tube in the Micro-Kolmer test for amebiasis, 0.2 units are thus contained per tube.

Complement titration

The complement titer was determined in 15/10 mm. tubes prior to the performance of each complement fixation test for amebiasis. The rack of tubes

and the reagents were kept in an ice bath. The complement diluted 1:40 and the Kolmer saline were delivered with 0.2 ml. pipettes (never measured with the tip of the pipette); the antigen and hemolysin-cells mixture, with 1 ml. pipettes.

2% sheep cells were sensitized with an equal volume of a dilution of hemolysin previously determined by hemolysin titration, 15 minutes before needed and placed in a refrigerator.

TABLE 1

Titration of complement

TUBE	COMPLEMENT (1:40)	KOLMER SALINE	ANTIGEN		SENSITIZED SHEEP CELLS	
	ml.	*ml.*	*ml.*		*ml.*	
1	0.10	0.05	0.10	Water bath at 37°C. for 1 hr.	0.10	Water bath at 37°C. for 30 minutes.
2	0.09	0.06	0.10		0.10	
3	0.08	0.07	0.10		0.10	
4	0.07	0.08	0.10		0.10	
5	0.06	0.09	0.10		0.10	
6	0.05	0.10	0.10		0.10	
7	0.04	0.11	0.10		0.10	
8	0.03	0.12	0.10		0.10	
9	0.02	0.13	0.10		0.10	
10	—	0.15	0.10		0.10	

TABLE 2

Dilution calculations for 2 exact units of complement to be contained in 0.1 ml.

TUBE WITH COMPLETE HEMOLYSIS	TWO EXACT UNITS OF COMPLEMENT	DILUTION RATIO
1	0.20	1:20
2	0.18	1:22.2
3	0.16	1:25
4	0.14	1:28.6
5	0.12	1:33.3
6	0.10	1:40
7	0.08	1:50
8	0.06	1:66.6
9	0.04	1:100

The usual dilution was 1:28.6 or 1:33.3.

The antigen stored in the deep freeze was placed at room temperature until almost completely thawed, then transferred to the refrigerator until needed.

When a new lot of antigen was used, the complement titration was set up with undiluted antigen and dilutions 1:2, 1:4, and 1:8 respectively, in order to establish the lowest dilution of antigen which was not anticomplementary.

The titration was set up as shown in Tables 1 and 2.

Titration of antigenic dose

The antigen was standardized by performing the Micro-Kolmer test described below with known positive and negative sera. Dilutions of antigen which gave

negative complement fixation tests with undiluted negative sera and the highest titers with positive sera were selected.

The lowest dilution of antigen fulfilling the above requirements was found to be 1:2.

Technique for test

TABLE 3

Preliminary dilutions of serum

TUBE	KOLMER SALINE	PATIENT'S SERUM	DILUTIONS
	ml.	*ml.*	
1	0.2	0.2 undiluted	1:2
2	0.2	0.2 from tube #1	1:4
3	0.2	0.2 from tube #2	1:8
4	0.2	0.2 from tube #3	1:16

1 ml. pipette was used to measure saline; 0.2 ml. pipette for serial transfers measured with the tip of the pipette.

TABLE 4

Final quantitative test

TUBE	PATIENT'S SERUM	KOLMER SALINE	TITRATED COMPLEM.	ANTIGEN		SENSITIZED SHEEP CELLS	
	ml.	*ml.*	*ml.*	*ml.*		*ml.*	
1	0.05 undiluted	0.10	0.10	—	Refrigerator overnight	0.10	Water bath at 37°C. for 30 minutes.
2	0.05 undiluted	—	0.10	0.10		0.10	
3	0.05 of 1:2	—	0.10	0.10		0.10	
4	0.05 of 1:4	—	0.10	0.10		0.10	
5	0.05 of 1:8	—	0.10	0.10		0.10	
6	0.05 of 1:16	—	0.10	0.10		0.10	
7	—	0.05	0.10	0.10		0.10	
8	—	0.15	0.10	—		0.10	
9	—	0.15	—	0.10		0.10	
10	—	0.25	—	—		0.10	

Tube #1 is patient's serum control for anticomplementary effect; it must be completely hemolysed.

Tube #7 is antigen control for anticomplementary effect; it must be completely hemolysed.

Tube #8 is hemolytic system control; it must be completely hemolysed.

Tube #9 is antigen control for hemolytic property; it must not be hemolysed.

Tube #10 is cell control; it must not be hemolysed.

Known positive and negative controls were included in every batch of tests.

The readings were compared with a color scale (Table 5) prepared by using different proportions of sheep cell dilution (1 part of 2% sheep cells + 6 parts of Kolmer saline) and hemolysed sheep cells (the sparkling tubes from hemolysin titration.

Interpretation of results

At least a four plus reaction in undiluted serum with lesser reaction in the diluted serum was considered a positive complement fixation test.

RESULTS

The Micro-Kolmer complement fixation test was performed in 303 cases. The results of these examinations are shown in Tables 6 and 7.

TABLE 5

Comparative hemolysis color scale used to read reactions of the complement fixation test

TUBE	SHEEP CELL DILUTION	HEMOLYSED SHEEP CELLS	READING
	ml.	*ml.*	
1	0.4	—	Four plus
2	0.2	0.2	Three plus
3	0.1	0.3	Two plus
4	0.04	0.36	One plus
5	—	0.4	Negative

TABLE 6

Results of the Micro-Kolmer complement fixation tests for amebiasis on a group of 303 individuals

CLINICAL DIAGNOSIS	MICROSCOPIC STOOL EXAMINATION	NUMBER OF CASES	POSITIVE MICRO-KOLMER COMPLEMENT FIXATION TEST
Intestinal amebiasis	*E. histolytica* trophozoites	22	20 (90.9%)
	E. histolytica cysts	7	2 (28.6%)
Gastro-intestinal disorders with no evidence of amebiasis	Parasites other than *E. histolytica*	120	4 (3.3%)
	No parasites in stools	98	7 (7.1%)
No gastro-intestinal disorders, no evidence of amebiasis	No parasites in stools	56	1 (1.8%)

Tables 6 and 7 show that among 22 cases harboring trophozoites of *E. histolytica*, 20 (90.9%) gave a positive Micro-Kolmer complement fixation test for amebiasis, while in 7 cases with only cysts of *E. histolytica* the Micro-Kolmer complement fixation test was found to be positive in only 2 cases (28.6%).

Among 120 cases harboring parasites other than *E. histolytica* only 4 were found to have a positive Micro-Kolmer complement fixation test for amebiasis (3.3%).

Of 154 individuals who did not reveal parasites in the stools on multiple examinations, 96 had gastro-intestinal disorders and 56 were control cases selected at random among the patients having positive serology for syphilis.

The symptoms of the 98 cases having gastro-intestinal disturbances ranged

TABLE 7

Results of the Micro-Kolmer complement fixation tests for amebiasis on a group of 149 individuals harboring parasites in stools

STOOL EXAMINATION	CASES	POSITIVE COMPL. FIX.
Single species of parasites		
Endamoeba histolytica cysts	5	2
Endamoeba histolytica trophozoites	8	7
Endamoeba histolytica cysts and trophozoites	4	4
Endamoeba coli cysts	18	0
Endamoeba coli trophozoites	8	0
Endamoeba coli cysts and trophozoites	1	0
Endolimax nana cysts	2	0
Endolimax nana trophozoites	1	0
Iodamoeba bütschlii cysts	1	0
Iodamoeba bütschlii trophozoites	2	0
Unidentified amebae	2	0
Isospora hominis oocysts	1	0
Giardia lamblia cysts	13	1
Giardia lamblia cysts and trophozoites	2	1
Trichomonas hominis	4	0
Schistosoma mansoni ova	7	0
Schistosoma japonicum ova	3	0
Hookworm ova	22	1
Trichuris trichiura ova	9	0
Ascaris lumbricoides ova	2	0
Strongyloides stercoralis larvae	8	0
Mixed infestations		
Endamoeba histolytica + Endamoeba coli cysts	1	0
Endamoeba histolytica + Endamoeba coli trophozoites	5	5
Endamoeba histolytica + Endolimax nana cysts	1	0
Endamoeba histolytica + Endolimax nana trophozoites	1	1
Endamoeba histolytica + Endolimax nana troph. + Hookworm o.	1	1
Endamoeba histolytica troph. + Strongyloides stercoralis l.	1	1
Endamoeba histolytica troph. + I. bütschlii cysts	1	0
Endamoeba histolytica troph. + Hookworm ova	1	1
Endamoeba coli + I. bütschlii cysts	1	0
Endamoeba coli + I. bütschlii trophozoites	1	0
Endamoeba coli + E. nana cysts and trophozoites	1	0
Endamoeba coli + E. nana trophozoites	2	1
E. coli troph. + T. trichiura ova + Strongyloides stercoralis l.	1	0
E. coli troph. + Strongyloides stercoralis larvae	1	0
Schistosoma japonicum + T. trichiura + Hookworm ova	1	0
Hookworm + Trichuris trichiura ova	2	0
Hookworm ova + Iodamoeba bütschlii cysts	1	0
Hookworm ova + Ascaris lumbricoides ova + G. lamblia cysts	1	0
Trichuris trichiura + Ascaris lumbricoides ova	2	0

from mild abdominal pain to severe dysenteriform attacks, with or without blood in the stools. This group was represented by cases which later were diag-

nosed as ulcerative and non ulcerative colitis, cancer of gastro-intestinal tract, hepatitis, pancreatitis and also disorders of unknown etiology. The sera of 7 cases of this group showed positive Micro-Kolmer complement fixation test for amebiasis (7.1%).

All 56 control sera had a positive Kolmer-Wassermann complement fixation test for syphilis, ranging from 1 to 512 Kolmer units. Of these, 55 (98.2%) showed a negative Micro-Kolmer complement fixation test for amebiasis. Nothing in the history of the one case showing a positive Micro-Kolmer test, suggested amebiasis.

DISCUSSION

Although the number of cases harboring *E. histolytica* in the stools was statistically insignificant, the 90.9% of positive Micro-Kolmer complement fixation tests in cases with trophozoites suggests that the test is sensitive in intestinal amebiasis. This sensitivity decreases markedly to 28.6% when only cysts of *E. histolytica* are found. The positive reaction in these cases suggests that, although clinically not significant, these were active cases in contrast to other cyst carriers with negative complement fixation tests.

On the other hand, the test seems to be quite specific, since in 274 cases not harboring any form of *E. histolytica*, only 12 showed a positive Micro-Kolmer test for amebiasis. These cases include one with a positive Kolmer test for syphilis (4 Kolmer units), four with intestinal parasites other than *E. histolytica*, and seven cases of gastrointestinal disorders of unknown etiology.

Not all of these twelve cases can deservedly be labelled "false positive" tests. Clinical evaluation of the case with positive serology for syphilis and the four cases with parasites other than *E. histolytica*, did not reveal anything that could point to latent amebiasis. Of the seven cases with gastro-intestinal disorders, however, two had a history of amebiasis, the clinical picture of two suggested a liver abscess, and in the other three, amebic colitis was suspected. In four cases antiamebic treatment consisting of emetine followed by diiodohydroxyquinoline (Diodoquin) or chloroquine, or both, was administered. One patient underwent a surgical intervention and a cyst (the nature of which was not determined) was excised from the lower lobe of the liver. Another case, which was diagnosed as a mechanical obstruction of the lower ileum, revealed upon operation pathology suggesting parasitic infestation. Five cases improved rapidly after antiamebic treatment and excision of lesions. Of these, the Micro-Kolmer complement fixation test for amebiasis became negative in two cases, the titers decreased in two cases and no change was observed in one. At least four out of the seven cases with gastro-intestinal disorders strongly suggested amebiasis despite the fact that *E. histolytica* could not be found in the stools. A condition which consists of localized amebic lesions without detectable amebae in the stools is more frequent than hitherto suspected. Ochsner and DeBakey (1943) report only 11.6% of stools positive for *E. histolytica* among 4,091 cases of amebic hepatitis collected from literature. During our sojourn in the Belgian Congo we came across quite a few cases of diffuse amebic hepatitis, frank liver

abscesses and amebic involvement of the lungs where no amebae could be discovered in the stools. In some cases *E. histolytica* was known to have been observed previously, but not detectable on numerous stool examinations during the time when it could be recovered from the lesions.

The reactions in the Micro-Kolmer test, considered to be positive, were arbitrarily selected. These reactions were decided upon because the titration of antigen revealed in no case of amebiasis harboring *E. histolytica* in the stools, with a positive complement fixation, a reaction of less than four plus in undiluted serum and lesser reactions in further dilutions. On the other hand, less than four plus in undiluted serum and the dilutions, or four plus in undiluted serum only, was found occasionally in negative control cases. This artificial division with a very small margin seemed to have been justified by the results obtained.

Similar results, with very slight variations in reactions could be obtained on repeat tests with two different lots of antigen 491 and 501 prepared by the method described above, both originating from strain 103 *E. histolytica*. It must be emphasized that both lots of antigen were produced from growths collected for months and which contained amebae of different stages, sizes and states. When antigen was produced from the same strain but collected for a short time by subculturing numerous tubes in one day from one tube, results with positive sera were not comparable to those obtained with the lots 491 and 501. Thus antigen lot 502 produced from cultures containing mostly large forms with pseudopodia, gave positive complement fixation tests with all available sera of the cases showing trophozoites in the stools, but exhibited much weaker reactions with sera from two cases harboring cysts only. These two sera gave significant reactions when tested with antigen lots 491 and 501. The antigen lot 504 from culture tubes containing only small rounded forms gave positive complement fixation tests in the same two cases harboring cysts of *E. histolytica*, but weak reactions were obtained with three sera showing strong reactions with all three antigen lots 491, 501 and 502.

Bozicevich (1950) demonstrated that the same sera could give a positive or negative complement fixation test for amebiasis depending upon which of his two antigens was used, emphasizing the necessity of using polyvalent antigen made from different strains of *E. histolytica*. He points to the apparent relationship between the small and large race of *E. histolytica* harbored by the patient, and the result of the complement fixation test.

The amebic size may not depend exclusively upon different strains, however. Meleney and Zuckerman (1948) reported a transformation in the culture of a small strain of *E. histolytica* into a large strain. It has been our experience that the incidence of small and large forms of *E. histolytica* depends upon the composition of the culture media.[3] In the present study, quite marked variation in size could be obtained in subcultures used for antigen production, even without modification of media. Although only four lots of amebic antigen produced from the same strain 103 were cross-checked with a very limited number of

[3] Unpublished data.

positive sera, there seems to be an indication that there may be a difference in antigen obtained from different stages and forms of one strain only.

SUMMARY

A Micro-Kolmer technique, modification of the Kolmer-Wassermann complement fixation method, for the detection of *Endamoeba histolytica* antibodies is presented.

A modification of the method for the production of antigenic reagent, which has been found to be specific and sensitive, is described.

Results on 303 cases tested for amebiasis with this method show that an amebic complement fixation test can be useful in intestinal amebiasis and in obscure cases in which no amebæ can be detected in the stools.

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A SEARCH FOR LIVER DAMAGE IN ANTI-AMEBIC TREATMENT WITH DIODOQUIN, VIOFORM AND CARBARSONE[1, 2]

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In recent years the internist has come to recognize that a clinical entity in the liver exists resulting from *Endamoeba histolytica* of the colon. The diagnosis of a low grade hepatitis is to be suspected in the presence of *E. histolytica* in the stools when the liver is enlarged and tender to percussion with low grade fever, a mild leukocytosis, and pain in the region of the liver. There may be only hepatic enlargement and tenderness without systemic response. As Sodeman (1950) pointed out, it is extremely desirable to treat these individuals who have hepatomegaly with colonic amebiasis before the development of an acute amebic hepatitis or hepatic abscess. Dennis (1950) reported 53 per cent of a group of 94 typical cases of subacute amebiasis of the colon with palpable livers.

Drugs used in the treatment of amebiasis must of necessity be more toxic to the amebae than to the host. Carbarsone and the halogenated hydroxyquinolines are usually effective in intestinal amebiasis but ineffective in hepatic amebiasis, probably because of inadequate concentration in the liver, which may be due to slow absorption from the small intestine, or detoxification of the drug by the liver. However, the authors (Knight *et al.* 1949) in 1948 found that, of the oxyquinolines diiodohydroxyquinoline (Diodoquin), iodochlorhydroxyquinoline (Vioform), and chiniofon (Anayodin), blood levels of Diodoquin and Vioform were sufficiently high in recommended therapeutic dosages to inhibit all growth of *E. histolytica in vitro* at the end of forty-eight hours and that this serum level, as measured in iodine, appeared to lie between 250 and 300 gammas of iodine per 100 ml. Eight subjects on Diodoquin after seven days had serum levels averaging 1143 gammas per 100 ml.

Because of the frequency of the tender and enlarged liver associated with colonic amebiasis, the question arose whether some of the widely used drugs had a further deleterious effect upon hepatic function. Reactions to carbarsone in the form of a dermatitis, diarrhea, and generalized aching are not infrequent. A sensitivity to Diodoquin in the form of a dermatitis is uncommon.

Four different liver function studies were performed on fifteen patients who had proven intestinal amebiasis but were normal and healthy in other respects. The cephalin-cholesterol flocculation test, the hippuric acid test, the brom-

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sulphalein excretion test, and the icteric index were done prior to any treatment, again after 21 days of Diodoquin, again following ten days of carbarsone, and

TABLE 1

Effect on hanger test

PATIENT	PRIOR TO TREATMENT		AFTER TREATMENT WITH DIODOQUIN		AFTER TREATMENT WITH VIOFORM		AFTER TREATMENT WITH CARBARSONE	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
1. P. C.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
2. A. G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	+2
3. E. P.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
4. N. F.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
5. J. R.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
6. A. M.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
7. P. V.	Neg.	+1	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
8. A. S.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
9. R. R.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
10. H. G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
11. F. D.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
12. F. M.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
13. B. A.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
14. P. W.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
15. M. G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

TABLE 2

Effect on Icteric Index

PATIENT	PRIOR TO TREATMENT	AFTER TREATMENT WITH DIODOQUĪN	AFTER TREATMENT WITH VIOFORM	AFTER TREATMENT WITH CARBARSONE
1. P. C.	9	14	6	8
2. A. G.	14	7	5	5
3. E. P.	14	13	12	13
4. N. F.	11	9	11	12
5. J. R.	10	11	7	7
6. A. M.	9	4	9	6
7. P. V.	8	11	8	12
8. A. S.	11	10	9	7
9. R. R.	10	10	8	14
10. H. G.	8	5	7	4
11. F. D.	10	7	9	8
12. F. M.	5	6	7	6
13. B. A.	5	5	5	4
14. P. W.	8	8	7	7
15. M. G.	7	7	6	6

again on completing ten days of Vioform. These drugs were given in the recommended therapeutic dosage. A total of 240 tests was done.

TABLE 3

Effect on bromsulphalein retention

PATIENT	PRIOR TO TREATMENT	AFTER TREATMENT WITH DIODOQUIN	AFTER TREATMENT WITH VIOFORM	AFTER TREATMENT WITH CARBARSONE
1. P. C.	0 %	0 %	10.8%	7.83%
2. A. G.	1.5	2.33	0	1.26
3. E. P.	12.4	3.72	0.8	6.96
4. N. F.	5.1	0	0.12	3.9
5. J. R.	2.4	3.84	0	0
6. A. M.	3.8	0	0	2.55
7. P. V.	0	5.13	3.5	0
8. A. S.	3.1	0	5.8	5.8
9. R. R.	0	3.65	0	1.6
10. H. G.	2.8	1.4	2.11	1.13
11. F. D.	0	0.80	0	0.444
12. F. M.	0	0	0	0.56
13. B. A.	2.6	1.5	0	0
14. P. W.	0	0	0	0
15. M. G.	0	1.5	0	1.40

TABLE 4

Effect on hippuric acid test

PATIENT	PRIOR TO TREATMENT	AFTER TREATMENT WITH DIODOQUIN	AFTER TREATMENT WITH VIOFORM	AFTER TREATMENT WITH CARBARSONE
1. P. C.	0.980 Gm.	1.1051 Gm.	1.382 Gm.	1.030 Gm.
2. A. G.	0.8876	0.737	0.671	0.834
3. E. P.	0.890	0.869	1.043	0.950
4. N. F.	0.889	1.158	1.12	1.098
5. J. R.	0.8905	0.889	1.067	0.861
6. A. M.	0.6658	0.729	1.272	1.082
7. P. V.	0.641	1.315	0.910	1.10
8. A. S.	1.235	1.145	0.992	0.943
9. R. R.	1.260	1.402	0.950	1.133
10. H. G.	0.921	0.893	0.330	0.04455
11. F. D.	0.518	0.916	0.940	0.432
12. F. M.	0.9997	0.846	1.020	0.960
13. B. A.	1.00	1.10	0.998	0.980
14. P. W.	1.10	1.00	0.950	0.945
15. M. G.	1.04	0.975	1.16	0.978

METHOD OF TESTS

The routine cephalin-cholesterol flocculation test of Hanger (1938) was performed with readings at the end of 24 and 48 hours.

Quick's (1938) intravenous hippuric acid test which is based upon both detoxifying and metabolic functions of the liver was done since it obviates the nausea

and vomiting which frequently accompanies the oral test. The normal excretion of hippuric acid should be about 1 Gm. in 1 hour.

In the determination of bromsulphalein in plasma (Rosenthal *et al.* 1925), a control blood sample was drawn just prior to injection of the dye; 45 minutes after the injection of 5.0 cc. of the dye, containing 250 mg. of bromsulphalein, a second blood sample was drawn for analysis of the dye content. More than 5 per cent dye retention is considered abnormal.

The icteric index was performed in the routine manner by comparison of the different serums.

It is recognized that because of the many physiologic functions and the great functional reserve of the liver, and the relative insensitivity of our present liver function tests, a certain degree of dysfunction and hepatocellular damage may be present due to colonic amebiasis or to the drugs that is not detected by laboratory tests.

It will be observed in Table 1 that the Hanger became positive in only two subjects and then only after 48 hours. The icteric index was either the same or lower after completion of treatment (Table 2). There was no significant retention of bromsulphalein at any time (Table 3). Hippuric acid excretion was in general normal throughout the study (Table 4).

CONCLUSIONS

Fifteen patients with laboratory proven intestinal amebiasis were treated with diiodohydroxyquinoline (Diodoquin), carbarsone, and iodochlorhydroxyquinoline (Vioform) over a period of six weeks. Four different liver function tests were performed on each subject prior to treatment and following the completion of each drug. There was no apparent damage detectable due to therapy. No studies of liver function were done with antibiotics in the treatment of amebiasis. The authors believe that carbarsone and the hydroxyquinoline drugs still remain the treatment of choice in amebiasis because of the disturbance of the normal intestinal flora of the colon with some of the newer antibiotics.

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SOME EFFECTS OF CULTURAL ASSOCIATES ON THE INFECTIVITY OF A STRAIN OF *ENDAMOEBA HISTOLYTICA* FOR THE RABBIT

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Although many of the common laboratory animals have proven to be susceptible experimental hosts for *Endamoeba histolytica*, the infections obtained have been somewhat diverse depending upon the species of animal and the strain of ameba employed (Craig, 1944; Neal, 1951). The type of bacterial associates with which the amebae are grown *in vitro* probably constitutes a major factor that should be considered when evaluating the infectivity and pathogenicity of strains of *E. histolytica* for animals. Recent improvements in isolation and cultivation procedures developed by Rees *et al.* (1941) and Phillips (1950) have provided means of controlling the associated organisms to the extent that a given strain of ameba may be grown with any number of known associates. In the studies herein described, these methods have been utilized to isolate a single strain of *E. histolytica* with various bacteria and with *Trypanosoma cruzi*. The resultant cultures have been tested to determine their infectivity and pathogenicity for the rabbit.

MATERIALS AND METHODS

The NIH strain 200 of *E. histolytica* was isolated by Tobie in 1947 (1949) at sigmoidoscopy of a patient with acute amebic dysentery. The material obtained was inoculated directly into a dog from which cultures were started; later, 1948, a passage through a rabbit was also made. After these animal passages the cultures were continued by serial passage in Locke's-egg-rice starch (LER) medium. This strain was then established with the associates listed in Table 1. Cultures of the amebae with organism *t* (culture *t*), and with NRS flora were first obtained in 1949 by the microisolation of cysts from cultures. Another culture R, was started in 1949 with scrapings from an ulcer in the intestine of a rabbit. The cultures of the amebae with *T. cruzi* (A-T) represent two isolations, 1950 and 1951, one obtained by the microisolation of trophozoites from culture R and the other by transfer of large numbers of trophozoites into A-T medium in which the bacteria were eliminated by the use of penicillin G and streptomycin and by serial transfer.

All cultures of amebae with the exception of A-T were transferred three times each week to fresh LER medium; the A-T cultures were maintained by the method of Phillips (1951). The A-T amebae were returned to culture with organism *t* (culture A-T-*t*) and to culture with the NRS flora (culture A-T-N) by transferring the A-T trophozoites to LER medium with the original bacterial associates.

In an attempt to increase the infectivity of the A-T amebae, the following

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modifications of the A-T inoculum were made before administration to two series of rabbits. Defibrinated rabbit blood was added to cultures during the week prior to inoculation, and, in another instance, heat-killed NRS bacteria were added two days before inoculation. Since there was a question about the influence of the bacterial flora of the cecum on the survival of A-T amebae, a third series of animals was prepared for the inoculation of A-T amebae by treatment with 50,000 units of penicillin and 0.5 gm. of sulfasuxidine given orally each day for four days while another series was given 10 mg./kg. of streptomycin intramuscularly twice daily for each of five days before inoculation.

The method of harvesting the ameba-bacteria cultures and of making inoculations has previously been described by Luttermoser *et al.* (1952). The A-T cultures were transferred to 50 ml. tubes and concentrated by centrifugation at 2,000 rpm for three minutes.

Young New Zealand white rabbits fed a deficient diet consisting of corn, oats, and bran were inoculated intracecally with approximately 200,000 amebae

TABLE 1

Amebiasis in rabbits inoculated with strain 200 E. histolytica *grown with various associates*

CULTURE INOCULUM	ASSOCIATES	NO. ANIMALS	NO. WITH ULCERS	NO. DYING OF AMEBIASIS	AVERAGE SURVIVAL PERIOD (A.S.P.)	AVERAGE DEGREE INFECTION (A.D.I.)
					days	
200 A-T	*T. cruzi*	24	1	0	—	0.04
200 *t*	Org. *t*	34	17	12	21.4	1.5
200 R	Rabbit cecum flora	126	106	104	16.0	3.25
200 N	NRS flora	91	84	78	14.6	3.14
200 A-T-*t*	Org. *t*	28	11	10	16.1	1.36
200 A-T-N	NRS flora	17	14	10	13.2	2.45

from the various cultures. In addition, the A-T trophozoites were also inoculated into the lower ileum or into the appendix of a small number of the animals. The animals were observed frequently for the development of diarrhea and were weighed at least once each week. If the animals did not develop fatal ulcerative amebiasis within 40 days, they were sacrificed and examined for the presence of cecal lesions containing amebae. The infections were graded 0, 1+, 2+, 3+, and 4+ according to the number, size, and extent of the lesions (Luttermoser *et al.*, 1952). The average degree of infection (A.D.I.) for each group was calculated from these data. The average survival period (A.S.P.) for each group was determined from the survival time of those animals dying of the disease.

RESULTS OF ANIMAL INOCULATION

Comparative results of the intracecal inoculation of rabbits with strain 200 of *E. histolytica* cultivated with various associates are summarized in Table 1. Fifty per cent of the animals which received the amebae from culture *t* became infected as compared to 92 per cent of those inoculated with culture N. The

results with amebae of culture R are similar to the latter. Further differences were apparent in that only 35 per cent of the animals inoculated with *t* amebae died from amebiasis, whereas about 85 per cent of those inoculated with the R and N cultures succumbed to the disease. The animals infected with the *t* culture survived on the average about one week longer than those given the R or N cultures, and the infections resulting from the *t* inoculation required a longer period to develop as evidenced by a comparatively late appearance of macroscopic ulcers in the cecal wall. Furthermore, the grades of the infections in the N and R series were found to be twice the degree of intensity of those in the rabbits of the *t* series.

Of 24 animals which had received the A-T amebae, only one showed an ulcer containing trophozoites. Upon examination of the large intestine of some of these animals four or more days after inoculation, trophozoites were found neither in the lumen nor in scrapings from the wall. In other tests 33 rabbits received treatment with antibiotics before inoculation with A-T amebae or received the A-T inoculum with modifications. The addition of blood or heat-killed bacteria to the A-T cultures failed to alter their infectivity. Similarly, the inoculation of amebae into other parts of the intestine and the pre-inoculation drugging of animals with antibiotics exerted no influence on the results. However, when the A-T amebae were returned to LER medium and were re-established with living bacterial associates, their infectivity was restored. Forty-five per cent of 28 animals inoculated with A-T amebae which had been returned to culture with organism *t* developed amebiasis. Furthermore, 82 per cent of 17 animals developed the disease when they were given A-T amebae returned to culture with the NRS flora. It is of interest that these infection rates were similar to those which resulted from inoculation of the original *t* and N cultures. Incidentally, the simultaneous administration of living NRS bacteria with the A-T amebae produced no infections in 6 animals. Rabbits inoculated with organism *t* or the NRS flora alone showed no ulceration.

In vitro OBSERVATIONS ON A-T AMEBAE

In view of the fact that the A-T amebae, with few exceptions, failed to become established in the intestine of the receptor animals, observations on the establishment of A-T amebae with bacterial associates *in vitro* are of interest. When approximately 25,000 A-T amebae were inoculated into each tube of LER medium with NRS bacteria, the amebae survived in large numbers for approximately 48 hours. Following this, the cultures were transplanted three times weekly, but for the first 2 to 4 weeks few viable trophozoites were found. Furthermore, some became entirely free of amebae. In three transfers of the A-T amebae to the LER medium with mixed bacterial flora, it required 14, 18, and 32 days, respectively, before the population in each tube reached 100,000 or more amebae. The great increase in numbers of these organisms occurred during the last few days of the periods mentioned, and only about 75 per cent of the tubes developed numerous amebae. A similar lag in adaptation *in vitro* was observed when the A-T trophozoites were placed in the LER medium with

organism *t*. However, it appeared that the amebae became more rapidly established with this bacterium.

DISCUSSION

It was found that the infectivity and pathogenicity of cultures of strain 200 *E. histolytica* varied depending on the cultural associates. When cultivated with the NRS or rabbit intestinal flora, trophozoites of the 200 strain of amebae produced acute disease in most of the inoculated animals. However, only half of the animals receiving an inoculum of amebae cultivated with organism *t*, and less than 5 per cent of those inoculated with amebae grown with *T. cruzi* (A-T amebae), developed amebiasis. Furthermore, the disease developed more slowly and to a lesser degree in the animals of the latter series. Unreported results of Taylor of this laboratory were similar in that only 4 of 27 guinea pigs which were inoculated with A-T amebae, became infected. However, it was found in the present study that after the A-T amebae were associated again with bacteria *in vitro* their infectivity was similar to that of the original cultures.

The basis for this influence of the different cultural associates upon the infectivity of amebae is unknown. With regard to the restoration of the pathogenicity of the A-T amebae, it has been reported that the infectivity and virulence of *E. histolytica* can be influenced by encystment. Deschiens (1939, 1941) reported a decrease in the infectivity of two strains of *E. histolytica* upon prolonged cultivation followed by a return of infectivity after induced encystment. Chang (1945) confirmed the findings of Deschiens and suggested that the basis for restoration of infectivity may lie in the fact that amebae produced from the cystic stage may be more adaptable to environmental changes. Perhaps the nuclear reorganization inherent in encystment may influence the vigor of the organism as has been shown by Calkins (1919) and Kidder *et al.* (1938) to be true for two species of free-living protozoa.

The different infectivity exhibited by amebae of strain 200 in culture with various single associates or with various mixed flora allows some conjecture along the same lines. Since examination of the intestine of some animals, sacrificed at various periods upward of four days after inoculation with A-T cultures, failed to demonstrate the presence of amebae, some agreement with the theory of Chang (1945) is indicated. A-T amebae, following prolonged cultivation *in vitro* in the absence of bacteria, appeared to be incapable of adaptation with sufficient rapidity to the environment of the cecum of the animals to initiate an infection. *In vitro* observations support this explanation in that a delay was observed in the establishment of the A-T amebae in bacterial cultures. After the readaptation of the A-T amebae to bacterial associates *in vitro*, infectivity and pathogenicity were found to be restored. The influence of encystation may have been operative in the present work since it is well known that the 200 strain of *E. histolytica* not uncommonly produces cysts when cultivated with a mixed bacterial flora.

Although the data presented herein have shown a very low rate of infectivity for A-T amebae, further studies may indicate conditions under which their

infectivity is increased. In this connection, observations on the effects of varying the nutrition of both the experimental hosts and the amebae would be of interest.

SUMMARY

1. Trophozoites from various cultures of strain 200 *Endamoeba histolytica* were evaluated as to their infectivity for rabbits.

2. When cultivated with a mixed bacterial flora, strain 200 produced a fatal ulcerative amebiasis in about 85 per cent of the inoculated animals as compared to 35 per cent when cultivated with a single bacterial associate, organism *t*.

3. Trophozoites from bacteria-free cultures, with *T. cruzi* as an associate, produced but one infection when inoculated into 24 animals.

4. The infectivity of trophozoites of *E. histolytica* grown with *T. cruzi* was restored after the amebae were re-established in LER culture with bacteria.

5. ***In vitro*** observations demonstrated that amebae grown in the absence of bacteria (amebae-*T. cruzi* cultures) became readapted to an environment with bacteria only with difficulty. Hence, this is offered as a possible explanation for the low infectivity of these amebae when inoculated intracecally into rabbits, although the possible influence of encystation on infectivity must be considered.

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EPIDEMIOLOGIC APPRAISAL OF MALARIA IN THE UNITED STATES DURING 1951

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The malaria appraisal program was initiated in 1947 by the Communicable Disease Center with the collaboration of certain States. As originally conceived the purpose of this program was to measure the actual incidence of malaria and to eliminate spurious reporting. In 1950 the appraisal program became an integral part of the malaria surveillance and prevention program of the Communicable Disease Center and State health departments concerned. Public Health Service medical and nurse officers were assigned to certain States[1] to appraise individual cases and deaths attributed to malaria, and to promote better diagnosis and treatment of the disease.

Detailed reports of the results of appraisals through 1950 have been presented elsewhere (Quinby 1950; Andrews, *et al.* 1950; Andrews 1951; Quinby and Welch 1952). This paper is concerned, primarily, with malaria attacks occurring during 1951.

During 1951 the nation reported 6,427[2] cases as compared to 2,227 for the previous year. This increase can be attributed to the return of military personnel from Korea. There were 4,115 cases recorded as of military or foreign origin, and 2,312 as civilian and unstated categories. Many of these civilian cases were from servicemen diagnosed in civilian diagnostic centers and from Korean veterans recently discharged. It was not until July that the introduced cases began to appear in the reports in large numbers. Through June the incidence of malaria had continued to decrease; only 737 cases had been reported, of which Texas reported almost one-half.

Table 1 shows by States the number of cases appraised during 1951, the number found positive in laboratories approved by the State, and the place where nfection was acquired. From 1,820 appraisals, a total of 1,232 parasite-positive cases were found to have had their onsets in 1951. Of these, 1,222 were acquired outside the United States; 1,212 from Korea, 2 from the South Pacific, 2 from Africa (West Coast and Belgian Congo), and 1 each from India, Italy, Liberia, Philippines, Cuba, and Colombia. There are only 10 cases in which there was no clear source of infection outside of the United States; 9 of these warrant summaries.

Arkansas Appraisal No. 394

B. E. S., a private stationed at Camp Chaffee, Arkansas, had been in the service less than 3 months when he became ill on August 31, 1951 with diurnal cycles

[1] Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, South Carolina and Texas have been thus assisted for some period during the last 4 years.

[2] Provisional and subject to correction.

of fever, splenomegaly, and slight jaundice. The patient had spent his entire life in Michigan except for a short trip to Alabama several years ago. On September 3 and 7, 1951 his blood was positive for *Plasmodium vivax*. These smears were confirmed by the Arkansas State Board of Health whose epidemiologist concluded that this case was of origin within the United States, most likely at Camp Chaffee, Arkansas.

Georgia Appraisal No. 6

The case was confirmed in the Georgia State laboratory August 15, 1951 as a *vivax* infection in a 40-year-old white male who had resided in Minter, Laurens

TABLE 1 *

Appraisal of malaria in the United States occurring in 1951*

STATE	APPRAISALS IN EACH STATE	CLASSIFICATION OF APPRAISED CASES			
		Positive in laboratory approved by State			All other
		Total	Acquired outside U. S.	Acquired within U. S.	
Alabama	131	62	62		69
Arkansas	241	184	183	1	57
Florida	33	18	18		15
Georgia	818	471	470	1	347
Kentucky	1	0			1
Louisiana	12	11	11		1
Mississippi	51	43	43		8
Missouri	7	7	4	3	
New York	1	1		1†	
South Carolina	380	365	364	1	15
Tennessee	144	69	67	2	75
Texas	1	1		1	
Total	1,820	1,232	1,222	10	588

* Through January 1952. The contributions of State, Public Health Service, and Tennessee Valley Authority epidemiologists are acknowledged.

† By transfusion (*P. malariae*).

County, Georgia, for the last 6 years. A record of illnesses during 4 years suggested malaria relapses. Two brothers, who lived with the patient in 1945 and 1946, were veterans from Panama. One of them had clinical malaria but had never shown a positive slide. The other brother developed rheumatic fever while in a highly malarious area of Panama, and since his return to the States he has been admitted on numerous occasions during the past few years to Dublin Veterans Hospital for treatment of this arthritic condition. No other positive malaria has been discovered in Laurens County since some time prior to 1948. The houses in the vicinity of this case had been sprayed with DDT twice a year, beginning in 1946. Blood films obtained from 53 persons living within 1½ miles of the patient were negative. The Georgia Department of Public Health considers this an old relapsing case probably dating back to 1948.

Missouri Appraisal No. 5.

W. S. M., a 26-year-old white male roentgenologist, was accidentally discovered to have malaria during the course of a blood examination for possible damage from excessive x-ray exposure. On September 27, 1951 a thin smear was found to be positive for *P. vivax*. The slide contained many plasmodia of all morphologies evenly distributed throughout the slide, thus ruling out the probability of mechanical transfer. About two weeks prior to this positive smear, the patient had headache, anorexia, and general malaise which became progressively worse. Although this physician took his temperature only once, there were no symptoms characteristic of fever. Treatment with chloroquine relieved the symptoms. This patient, a resident of Pennsylvania, had been stationed at Camp Atterbury, Indiana, in the fall of 1950 before being transferred to Fort Leonard Wood, Missouri. On May 6, 1951 he drove leisurely through Forest Park in St. Louis for about 30 minutes between 3 and 4 p.m. The lakes in this park are believed to have produced the malaria vectors causing one other case (M. H. No. 6) on August 1, 1951. The Missouri Department of Public Health and Welfare has attributed this case to Fort Leonard Wood.

Missouri Appraisal No. 6

M. H., a 73-year-old white female, had lived in St. Louis for 30 years and had never left Missouri in her life. During the last two years she had not been away from the city of St. Louis except for two short recreational trips of less than 15 miles. A blood smear submitted by the Jewish Hospital was confirmed by the National Depository as *P. vivax*. Onset of illness was August 1. Although the patient gave a history of malaria many years ago as a young girl in Missouri, there has been no notable illness in recent years except for an attack of pneumonia.

Interestingly enough, and fortunately from the standpoint of determination of the source of this case, the patient is an elderly maiden who left her home very infrequently. Her sister stated that they had lived "pretty much in a groove" in and about their home, which is two and one-half blocks from Forest Park, in the western part of the city. A chain of lakes in the park, one of which is less than one-half mile from the patient's home, provided a considerable acreage of *Anopheles quadrimaculatus* breeding areas. *A. quadrimaculatus* station counts in the park were as high as 77 in August and September. The patient's family had complained of being bitten all summer long while on their porch at night. The populace frequented evening functions in the park. Men of the Armed forces, home on leave or from nearby Army installations, were numerous among these summer crowds.

This case has been appraised as a primary attack of *P. vivax*, the source of which was either in St. Louis or St. Louis County, the most likely carrier being an infected serviceman from Korea.

Missouri Appraisal No. 7

G. J. F., a 22-year-old white male carpenter, entered the army on March 22, 1951 in Maryland, was transferred to Camp Pickett, Virginia for approximately

3 months, and subsequently to Fort Leonard Wood, Missouri. On July 8, 1951 he developed a fever of 104.4°F. but had no chills. On July 9 his blood was positive for *P. vivax*. The patient had never been away from his home in Pennsylvania except for the above described service during 1951 in Maryland, Virginia and Missouri. Because of the large numbers of the Korean cases active at Fort Leonard Wood and the presence of *Anopheles* mosquitoes, the Missouri State epidemiologist appraised this case as a primary attack, of origin in Fort Leonard Wood.

South Carolina Appraisal No. 77

Sergeant B. P., a 34-year-old white male, developed typical symptoms of malaria on August 6, 1951. A blood smear was found positive for *P. vivax* by the Fort Jackson dispensary on the same date. The Sergeant had not been to Korea although he had been in Egypt, India, and China during World War II, but with no known malaria during that time. He gave a history suggestive of malaria in 1933, 1936 and 1937. On June 16 and 18, 1951 he had fever and chills which were controlled with aspirin, but no antimalarial drugs were given.

The Sergeant frequently traveled to his home in Cleveland, Mississippi, while on leave, including periods before and after each of the bouts of fever in June and August. He usually spent 4 or 5 days out of each week on or about Fort Jackson in Richmond County, South Carolina. Sixteen days before the onset of his only confirmed attack of malaria, the patient spent 2 hours (part of it after dark) at Aqua Prieta, Mexico, a border town opposite Fort Huachuca, Arizona.

The South Carolina State Board of Health has designated this case as a first relapse of a 1951 primary attack, with the following possible sources of origin by order of likelihood: (1) Richmond County, South Carolina, (2) State of Mississippi, and (3) Aqua Prieta, Mexico.

Tennessee Appraisal No. 45

A 52-year-old colored male resident of Memphis, Tennessee, died on July 30, 1951 with final diagnoses of lymphoid leukemia and quartan malaria. Within 24 hours after hospital admission on July 24, a *Plasmodium malariae* parasitemia was discovered. There was no history of blood transfusions, no past history of syphilis, nor had the man ever been a patient in a mental hospital. However, the State Health Department expressed some concern that possibly one of the many discharged paretic patients from the Gailor Psychopathic Institute, who live near the Wolf River in North Memphis, may have served as the source of this case. It is also possible that this may have been an old infection with preagonal activation.

Tennessee Appraisal No. 46

An illness in a 28-year-old white male resident of Collierville, Shelby County, Tennessee, was diagnosed as *P. vivax* with onset on July 4, 1951. The slide was confirmed by the Tennessee Department of Public Health and was sent to the National Depository. In different interviews with investigators from Tennessee and Mississippi the patient gave varying histories which led to some doubt as to

their reliability. The patient claimed that 15 years ago he had malaria which was promptly relieved by quinine, and that there were no recurrences. He was a veteran of World War II, having served in England, France, and Germany without a history of malaria. There was no history of transfusions or injections, nor was there evidence that malaria mosquitoes were to be expected around his home. The patient stated that within 3 months before onset he fished at many locations in Tennessee and at several sites on the Sardis Reservoir in Mississippi. According to the Tennessee Department of Public Health, as far as the investigations have gone, the origin of this case was from an undetermined place within the United States.

Texas Appraisal No. 1

J. R., a 45-year-old white male farmer, had an onset of chills and fever with paroxysms occurring in August every 2 days. He was treated at home with an antimalarial drug with a good response and no subsequent symptoms. Blood slides made on September 6, 1951 were positive in the San Benito-Cameron County Health Unit Laboratory for *P. vivax*. This resident of the Rio Grande Valley, who lived about 20 miles from the river, had not been out of the United States "*this year*." Data on travel outside of the United States prior to 1951 were not available. However, he made weekly trips to an area below the Brownsville airfield to secure Mexican labor. On a basis of data presently available, the Texas State Department of Health concluded that the source of this infection was the Rio Grande River region.

TABLE 2

Appraisal of malaria cases reported or discovered in 13 eradication states

MALARIA CASES	1948	1949	1950	1951
Reported	9,317	4,012	2,099	3,915*
Appraised	770	514	690	1,819
Positive†	242‡	62	30	1,231
Primary	Undetermined	17	6	8
Relapses		8	6	1
Introduced		29	13	1,222
Transfusion		2	1	0
Induced		1	2	0
Source unknown		5	2	0

* Provisional and subject to correction.

† By year of attack.

‡ Also includes "Presumptives" i.e., with consistent clinical histories but not confirmed by approved laboratories.

DISCUSSION

Of the 1,232 positive appraisals, the species of *Plasmodium* were indicated in 1,079. *P. vivax* was the predominant parasite. Only 10 *P. malariae* were discovered, of which 4 were mixed infections with *P. vivax*.

Of the 588 attacks appraised as other than positive, it is believed that more

than two-thirds might actually be cases of introduced malaria. For instance, there were 403 cases in Korean veterans included in this category because the blood-positive confirmations were not obtained from laboratories approved by the State health departments. However, the data on the remainder of this group do not indicate that substantial numbers of *bona fide* cases exist.

For a comparison by years, Table 2 summarizes the results of appraisals in relation to reports[3] in the 13 eradication States.[4] The indigenous primary cases decreased from 17 in 1949, to 6 in 1950, and increased to 8 in 1951. During 1951 introduced cases increased manyfold.

It will be seen in the case abstracts that five States, Arkansas, Missouri, South Carolina, Tennessee, and Texas, claim to have had primary malaria cases during 1951. Infections in servicemen living on military bases where Korean cases were numerous were discovered in three of these states: Arkansas, Missouri, and South Carolina. Consequently it was concluded that the most probable source of these cases was the establishment of Korean strains in residents of this country. One case (M. H. No. 6) from Missouri occurred in a resident whose only association with known infection was in a park in St. Louis.

It would appear from the foregoing findings that it will be 3 years or more before five of the 13 traditionally malarious States will be able to comply with the criterion of cessation of endemicity of malaria defined by the National Malaria Society (Hinman 1951).

CONCLUSIONS

1. Except for the increases due to service personnel returned from Korea, reported malaria morbidity has continued to decline in the nation during 1951.

2. Epidemiologic appraisal of malaria occurring in 1951, in 11 of the 13 eradication States, showed 9 parasite-positive cases in which no clear source of natural infection outside the United States was discovered, 8 of these being regarded by State epidemiologists as primaries and the other as a relapse. The primary cases occurred in five states: Arkansas, Missouri, South Carolina, Tennessee, and Texas.

3. In Arkansas, Missouri, and South Carolina, one or more primary cases in United States residents were believed to have originated from Korean cases associated in time, place, and person on military bases. In Missouri 3 primary cases were discovered, 2 on military bases and 1 in a civilian in St. Louis.

4. Five of the 13 eradication States will be 3 years or more in meeting the criterion of cessation of endemicity of malaria defined by the National Malaria Society.

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[3] The failure of case numbers in Table II to agree with those cited in the references is due to additional available data since these papers were published.

[4] Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Oklahoma, South Carolina, Tennessee, and Texas.

partment of Public Health and Welfare, Dr. C. B. Tucker, of the Tennessee Department of Public Health, and to members of the Malaria Control Division of the Tennessee Valley Authority, for their extensive cooperation in the appraisal program, without assistance of epidemiologic personnel from the Communicable Disease Center.

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DENSITIES OF *ANOPHELES*, SPOROZOITE RATES IN *ANOPHELES*, AND INCIDENCE OF MALARIA IN AN AREA OF SOUTH CAROLINA

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During the years 1947 to 1950 inclusive, blood-positive cases of malaria disappeared from a formerly endemic area bordering the Santee Reservoir in Clarendon County, South Carolina. Anopheline sporozoite rates and densities for this period were observed and are presented here.

Previously, Sabrosky, McDaniel, and Reider (1946) noted a high infection rate in *Anopheles crucians* Wiedemann in addition to the recording of infective *Anopheles quadrimaculatus* Say in the community. Weathersbee and Frohne (1948) investigated the relationship between infectiveness and densities of *A. quadrimaculatus* in the same locality. Under existing conditions at that time, these authors could not determine the minimum density of this mosquito below which malaria transmission would not be expected to occur. Frohne, Weathersbee, Williams, and Hart (1950) reported continuing infections of *Plasmodium* in anophelines in the area in 1947 and 1948 despite the relatively low incidence of human malaria. Since this last publication, additional observations have accumulated.

METHODS

A monthly survey of the 2,000 people in the study group has been made for more than 5 years. Thick blood smears have been taken routinely from 85 to more than 90 per cent of this population.

Approximately 30 index and collecting stations have been utilized in the anopheline investigations. Weekly counts of female *Anopheles* in occupied stables largely furnished the basis for the determination of average densities in the experimental area. A total of 17 of the stations has been used during all the observations. Other stations were chosen from year to year to supplement the basic 17.

Anophelines for dissection were collected by means of suction tubes and were brought into the laboratory in modified cardboard mailing cylinders. Because of climatic conditions, practically all the collections were made from early April until late October, since it was during this time that the mosquitoes were present in appreciable numbers. Every effort was made to examine the salivary glands within 3 days to detect sporozoites. The term "sporozoite rate" refers to the percentage of positive mosquitoes based on the total dissected.

OBSERVATIONS

Considerably more than 20,000 human blood films were examined during each of the years 1947 to 1950 inclusive, as shown in the table. From 97 positive

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smears, or 0.39 per cent, in 1947, the number dropped to 9, or 0.04 per cent, in 1948 and declined to 1, or 0.01 per cent, in 1949. No positives were observed in 1950.

Information concerning dissections and densities for *A. crucians* and *A. quadrimaculatus* is also given in Table 1. These two species are the chief anophelines present, although *Anopheles punctipennis* Say is found in very sparse numbers in this locality.

The number of females of *A. crucians* dissected annually varied approximately from 5,000 in 1947 to 15,000 during 1949. A total of 34,808 was observed over the 4-year period, 1947 through 1950. Thirty-one specimens, or 0.09 per cent,

TABLE 1

Summary of Blood Film Examinations, Anopheles *Data, and Dissections of* Anopheles crucians *and* Anopheles quadrimaculatus *1947–1950*

EXAMINATIONS	YEAR			
	1947	1948	1949	1950
Blood films				
No. examined	24,575	23,306	21,928	21,896
No. positive	97	9	1	0
Percent positive	0.39	0.04	0.01	0
Mosquitoes				
A. crucians				
Average No. females per station	6.8	12.9	13.8	2.6
No. dissected	5,138	8,394	14,818	6,458
No. gland-positive	8	5	14	4
Percent positive	0.16	0.06	0.09	0.06
A. Quadrimaculatus				
Average No. females per station	2.5	15.0	10.8	1.6
No. dissected	2,127	8,473	6,365	3,138
No. gland-positive	2	3	5	0
Percent positive	0.09	0.04	0.08	0

were found infected as shown in the table; there was some variation from year to year in the rate of positive findings.

Dissections of *A. quadrimaculatus* ranged from slightly more than 2,000 in 1947 to nearly 8,500 the following year, and over four years, including 1949 and 1950, 20,103 individuals of this species were examined. There were 10 females, or 0.05 per cent, noted with sporozoites in the salivary glands. Here again, variations occurred in the yearly sporozoite rates from 0.09 per cent in 1947 to zero in 1950.

Although data compiled during 1951 are not detailed in this report, positive specimens of *A. crucians* and *A. quadrimaculatus* were recorded in the studies this year.

The number of infective anophelines collected during any single month or from any one particular station was not statistically significant. The same was

true with yearly comparisons. No significant differences were observed between the numbers of gland infections noted in *A. quadrimaculatus* and in *A. crucians*.

Numerous attempts to infect human volunteers and certain birds such as canaries and young domestic chickens by inoculations with the unknown sporozoites from the wild-caught anophelines were unsuccessful.

Densities given in the table were computed as average weekly counts of female anophelines at the index stations throughout the designated year. In the case of *A. crucians*, the density was lowest in 1950 (represented by a figure of 2.6), and highest in 1949 when the average was 13.8. *A. quadrimaculatus* reached a low of 1.6 in 1950 also, but the greatest density, 15.0, was recorded during 1948.

DISCUSSION

Apparent differences in the occurrence of sporozoite-laden anophelines both as to time and place did not prove significant when analyzed statistically. Whereas the number of malaria-positive blood films dropped to, and remained at, zero, the rate at which the infective mosquitoes were found did not vary significantly. This was true even in the case of *A. quadrimaculatus* in 1950 when no females with sporozoites were recorded. The per cent observed to be infected in previous years was so low that completely negative dissections for an entire season were not unexpected. From another standpoint, it may be important that sporozoites were again observed in specimens of *A. quadrimaculatus* in 1951. Attention is called to that particular fact, since this mosquito was always considered the foremost vector of human malaria in the area.

There was no correlation evident between the densities of the anophelines and the incidence of positive human blood films. In 1949, when only 1 smear was observed to contain malaria parasites, approximately 4 times as many *A. quadrimaculatus* and twice as many *A. crucians* were taken as in 1947 when nearly 100 positive slides were found.

CONCLUSIONS

1. The disappearance of malaria-positive human blood films from a study area bordering the Santee Reservoir in Clarendon County, South Carolina, was not accompanied by a significant decline in the sporozoite rates of anopheline mosquitoes during the years 1947 through 1950.

2. No significant differences were observed as to times and places of collections of the positive specimens of *A. quadrimaculatus* or *A. crucians*.

3. The identity of the sporozoites noted in the wild-caught anophelines is unknown. A number of inoculations using human volunteers and birds were attempted without subsequent infections.

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SYNERGISTIC EFFECT OF *HAEMOBARTONELLA MURIS* ON *PLASMODIUM BERGHEI* IN WHITE RATS[1]

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HISTORICAL REVIEW

In 1946, Vincke found sporozoites of an unknown *Plasmodium* in the stomachs of *Anopheles dureni* which were caught in a forest near Elizabethville in the Belgian Congo. At the same time, mammalian erythrocytes were also found in the contents of the mosquitoes' stomachs, suggesting the existence of a new malarial parasite. In 1948 two wild tree rats, *Thamnomys surdaster surdaster*, from this forest were found to harbor endoerythrocytic parasites, but the identity of these parasites could not be proven. After a careful study of the parasites in both the original hosts and other susceptible rodents, their distinctive morphology and course of infection warranted the creation of a new species, *Plasmodium berghei* (Vincke and Lips, 1948). The study was continued by various people in *Thamnomys surdaster surdaster* (Vincke and Van Den Bulcke, 1949c) and in cotton rats (Rodhain, 1949). In 1948, Vincke and Lips tried to infect *Anopheles gambiense*, *A. coustani*, *A. maculipalpis*, and *A. funestus* by feeding them on infected mice, but the results were negative. Raffaele and Baldi (1950) attempted to infect two additional species, *Aedes aegypti* and *Culex pipiens*, by the same procedure with equally negative results. However, in 1950, Vincke and Lips transmitted *P. berghei* by *Anopheles dureni;* and in 1951 Rodhain and Vincke studied the development of *P. berghei* in *Anopheles maculipennis* (var. *atroparvus*). Yoeli and Wall (1952) extended the study of the sporogonic development of *P. berghei* in *Anopheles stephensi*, *A. maculipennis* (var. *atroparvus*) and *A. quadrimaculatus*.

When this parasite from the wild rodents was inoculated into laboratory animals, mice and rats developed a fatal infection but guinea pigs and rabbits were resistant to infection (Vincke and Van Den Bulcke, 1949b; Galliard, 1949). Adler, Yoeli, and Zuckerman (1950) found *P. berghei* infective also for the golden hamster, *Mesocricetus auratur*, and for the field mole, *Microtus guntheri*. In the former host *P. berghei* produces a fatal infection in a high percentage of animals within a period of eight to twenty-nine days after inoculation, with a parasitemia up to 50 per cent of circulating red cells. However, in the latter host, *P. berghei* is nonpathogenic, producing only a transient patent infection with a parasitemia of 0.3–10 per cent and occasionally up to 30 per cent of red cells. The patent period in the field mole lasts about two weeks and the infection then becomes latent. Nevertheless after splenectomy the development of a high parasitemia is

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generally followed by death. Durand and Mathius (1951) reported the increase of virulence of the strain by a series of passages through hamsters as reflected in the earlier death of the animals (about a fortnight instead of three weeks).

Galliard and Lapierre (1950b), Schneider and Schneider (1950), and Mercado and Coatney (1951a & b) elaborated the study of blood-induced infections of *P. berghei* in white rats and white mice. Galliard and Lapierre (1951) showed that the mortality rate in white rats of 50 grams and above was 50 per cent, while that of 120 grams was less than 25 per cent. Splenectomy caused 100 per cent mortality in young rats, but rats of 100–250 grams survived. Van Riel (1950) tested the suitability of fruit bats, *Roussettus leachi*, as experimental hosts of *P. berghei* and found their parasitemia similar to that of the cotton rat. Vincke and Van Den Bulcke (1949a) also made some observations in respect to the course of disease, the susceptibility of the infection, and the results of the medication during the maintenance of the stock of K 173 of *P. berghei* in the laboratory, while Schneider, Decourt, and Montezin (1949) studied the chemotherapy of *P. berghei*.

In 1950, van den Berghe, Vincke, and Chardome studied the tissue phase of *P. berghei* and found that schizonts are formed in the cells of liver thirty-six hours after intraperitoneal inoculation, while blood-stream infection appears about forty-eight hours after inoculation. Therefore, the life cycle of *P. berghei* seems to follow a pattern similar to that of other mammalian malaria parasites.

PURPOSE OF THE PRESENT INVESTIGATION

The present investigation started as a study on drug resistance and gametocyte production as induced in malaria parasites by chlorguanide hydrochloride (Paludrine). Initially, *Plasmodium knowlesi* and *P. cynomolgi* were to be used for the studies, but *P. berghei* was chosen instead for economic reasons and because a greater volume of experiments could be performed.

At the time this work was begun, no detailed quantitative information about the course of infection with *P. berghei* in laboratory animals was available. Before the studies on drug resistance and gametocyte production could be started and correctly interpreted, it became necessary to determine the best susceptible host for our purposes, the size of inoculum, the course of infection, the degree of parasitemia, the gametocyte production, and the mortality rate. During the course of experiments to gain such information, the synergistic effect of *Haemobartonella muris* on the course of *P. berghei* infection in white rats was discovered. This finding complicated and lengthened the study because of the necessity of finding and rearing a *Haemobartonella*-free colony of rats for control experiments.

The results are reported here because of fundamental interest in antagonism and synergism of parasites for each other in the same susceptible host.

MATERIAL AND METHODS

The present strain of *P. berghei* was received by one of us (Q. M. G.) in October, 1950, through the courtesy of Dr. Willard H. Wright and Dr. G. Robert Coatney, of the National Institutes of Health, under Permit No. 147 from the U. S. Department of Agriculture. The origin of the strain (KBG 173) has been described by Mercado and Coatney (1951b).

For comparative purposes, the infection after intraperitoneal inoculation in white rats, mice, and hamsters was studied. Rats inoculated in the tail vein proved to be the most satisfactory hosts for our studies because of the course of parasitemia and the low percentage of extracellular parasites.

During the study, a total of 136 rats, 58 mice, and 12 hamsters were used. The rats were obtained from various sources (Sprague-Dawley, Carworth Farms, and local dealers) for experimental purposes and for a *Haemobartonella*-free strain. Splenectomy was performed on two strains of rats (Sprague-Dawley and Carworth Farms) to prove that they were free from *Haemobartonella*. Several litters of rats were raised from these splenectomized adult rats and were used for inoculation. At the same time, a number of passages of *P. berghei* were made from rats to hamsters, from hamsters to hamsters, and then from hamsters back to the *Haemobartonella*-free rats. The rats varied in weight from 40 to 300 grams, but the majority used weighed 60–100 grams, because this weight range gave consistent courses of infection.

As a routine method, the tip of the tail of the rat was clipped to get blood for a red blood count and blood films. Both thick and thin smears were made on the same slides, and thin smears were made on cover glasses. Giemsa stain and buffered water (pH 7.3) were used with 4.5 per cent Giemsa for forty-five minutes (this length of time is required to stain *H. muris* with certainty). A sample of 1000 erythrocytes was examined from each thin smear to arrive at (a) the percentage of erythrocytes infected, (b) the number of parasites per 100 erythrocytes, and (c) the percentage of various stages of parasites classified as prering, young trophozoite, old trophozoite, schizont, segmenter, gametocyte, degenerated, or extracellular parasite.

COURSE OF INFECTION

Various dosages of *P. berghei* were used in the test animals ranging from 6 million to 600 million parasites per kilogram of body weight. The most satisfactory inoculum for our purposes proved to be 60 million parasites per kilogram injected through the tail vein. This dosage gave a uniform incubation or prepatent period and curve of parasitemia in white rats averaging 100 grams in weight (Table 1). With this dosage, the incubation period in thirty-five rats averaged four to five days with the following range: 1 rat (2.8%)—9 days, 1 rat (2.8%)—6 days, 10 rats (28.5%)—5 days, 15 rats (42.8%)—4 days, 6 rats (17.1%)—3 days, and 2 rats (5.7%)—2 days. The average incubation period could be shortened or lengthened by increasing or decreasing the inoculum. The incubation period with an inoculum of 300–600 million parasites per kilogram was 2–3 days, but with a dosage of 6 million parasites per kilogram, the average incubation period was 5–6 days.

AGE SUSCEPTIBILITY

Although the three species of animals tested developed infection with *P. berghei*, the degree of parasitemia and mortality depended upon the age of the animal and dosage. Young animals varying from 60 to 100 grams were particularly susceptible, developing a high parasitemia that was generally followed by death

TABLE 1

Course of Plasmodium berghei *infection in white rats with various dosages and in the presence of* Haemobartonella muris

RAT		INOCULUM DOSAGE/KG. AND ROUTE	INCUBATION PERIOD	PATENT PERIOD	HIGHEST PARASITEMIA OCCURRED			DEATH	CONTROLLED INFECTION
No.	Wt.				Day	Percentage	Parasites per per cu. mm.		
	gm.		*days*	*days*				*days*	*days*
12	65	6 M.—i.p.	4	23	16	146.0	2,219,200		27
22[1]	65	6 M.—i.p.	7	9	5	20.9	875,760	16	
28[3]	60	6 M.—i.p.	3	14	14	149.0	2,711,800	14	
29[3]	60	6 M.—i.p.	7	15	18	151.0	2,521,700	21	
16[4]	65	600 M.—i.p.	2	6	6	55.8	998,820	7	
56[5]	80	600 M.—t.v.	3	13	13	147.0	1,955,100	14	
57[6] ♀	90	600 M.—t.v.	4	16	11	33.6	1,727,040		21
58[7]	90	600 M.—t.v.	3	11	7	41.1	1,109,700	15	
51	200	60 M.—t.v.	5	22	7	6.4	375,000		23
52 ♀	200	60 M.—t.v.	5	22	4	10.8	595,080		23
53	300	60 M.—t.v.	5	10	7	2.5	210,750		11
79	100	60 M.—t.v.	4	10	10	138.0	1,656,000	16	
80	120	60 M.—t.v.	5	14	14	142.5	2,166,000	20	
81	120	60 M.—t.v.	4	11	11	160.0	3,408,000	16	
85	58	60 M.—t.v.	4	7	6	106.0	2,067,000	11	
86	60	60 M.—t.v.	4	15	11	158.6	3,045,120	19	
88	70	60 M.—t.v.	4	7	7	191.0	2,320,200	11	
97	150	60 M.—t.v.	5	38	15	83.4	1,876,500		43
98	150	60 M.—t.v.	4	21	7	7.0	331,100		26
101 ♀	154	60 M.—t.v.	9	24	4	5.6	375,200		37
102	65	60 M.—t.v.	4	20	6	30.0	1,333,200		27
103	65	60 M.—t.v.	2	15	12	146.8	2,100,000	15	
108 ♀	70	60 M.—t.v.	4	14	7	57.2	1,430,000		17
111	76	60 M.—t.v.	4	10	10	156.5	1,611,950	11	
115	100	60 M.—t.v.	2	21	13	70.8	1,437,240		27
116	100	60 M.—t.v.	3	20	13	103.2	1,794,680		25
120	104	60 M.—t.v.	5	15	11	175.5	2,176,200	20	
126	77	60 M.—t.v.	3	16	6	101.0	3,080,000	18	
128	120	60 M.—t.v.	6	10	9	53.1	1,778,850	16	
139 ♀	75	60 M.—t.v.	5	15	5	9.5	536,750		20
141	95	60 M.—t.v.	3	12	12	185.5	2,504,250	13	
148 ♀	145	60 M.—t.v.	5	22	6	7.4	483,960		23

♀ = Female. All other rats were male.

i.p. = Intraperitoneal injection.

t.v. = Tail-vein injection.

[1] On the 11th day after inoculation, 3 cc. heart blood was taken for inoculation.

[3] Used for paludrine experiment. Paludrine was given on the 11th and 12th days.

[3] Used for paludrine experiment. Paludrine was given on the 16th, 17th, and 18th days.

[4] Used for inoculation on the 6th day and died.

[5] Used for paludrine experiment. Paludrine given orally on the 9th and 10th days.

[6] Used as control in paludrine experiment. 5 cc. of monkey's normal plasma was given i.p. on the 9th day.

[7] Used for paludrine experiment. 5 cc. of monkey's paludrine plasma was given i.p. on the 9th day and 3.8 cc. on the 10th day.

(Table 1). Animals weighing over 150 grams either died with lower parasitemia or acquired immunity to control the infection (Fig. 1). Young hamsters under 70 grams in weight were susceptible to infection, but older hamsters failed to develop

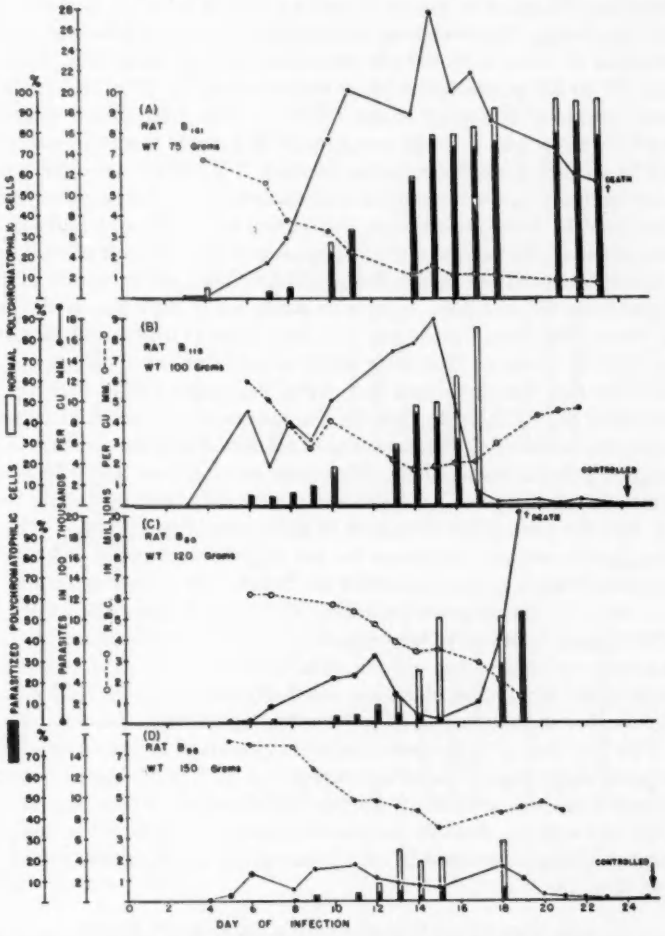

FIG. 1. Age susceptibility of white rats for *Plasmodium berghei*. Marked differences in rates of parasitemia and production of polychromatophilic cells are to be seen. With the exception of rat D, rats A, B and C showed a light infection of *Haemobartonella muris*.

detectable parasitemia regardless of inoculum used. Mice that were two to three months old showed a low infection rate or failed to develop detectable parasitemia. Of these mice, those that died showed a low grade parasitemia (less than 5

per cent) with heavily pigmented parasites. The younger mice after varying dosages frequently developed a parasitemia that exceeded 100 per cent, due to multiple infected erythrocytes. The course of infection in rats proved to be more consistent, and experiments with rats weighing 60 to 300 grams were carried out. Rats weighing 100 grams or less developed a course of infection that was dependent upon the dosage. Rats weighing 60 to 100 grams almost invariably developed a parasitemia of more than 100 per cent, followed by death (Fig. 1(A)). Rats weighing 100 to 120 grams had a lower rate of mortality (Fig. 1(C)); and a few recovered, acquiring immunity to the infection (Fig. 1(B)). Rats weighing 150 to 300 grams developed a lower parasitemia and made a spontaneous clinical recovery by acquiring immunity to the infection (Fig. 1(D)). Acquired immunity in this age group of rats was so strong that reinoculation with massive dosages of parasites failed to induce reinfection, confirming the work of Corradetti (1950). Thus, the severity of infection with *P. berghei* in white rats was more dependent on the age of the rats than on the dosage. The average patent period in younger rats ranged from ten to fifteen days, with death occurring between the eleventh and the twenty-fifth day. Vincke and Van Den Bulcke (1949b) and Mercado and Coatney (1951b) observed that their white rats died between eleven and fifteen days. In older rats that developed immunity, the patent period varied from ten to thirty-eight days (Table 1). During the course of this malarial infection in white rats, the outstanding characteristic of the blood was the presence of a high percentage of polychromatophilic erythrocytes ranging from 28 to 96.5 per cent (Fig. 1(A)). Great numbers of polychromatophilic cells contained more than one parasite, but the percentage decreased in older rats (Fig. 1(C) and 1(D)). The parasites showed marked preference for the polychromatophilic cells, and very few parasites invaded mature erythrocytes. Sometimes, even the nucleated erythrocytes revealed one or more parasites. Multiple infection of erythrocytes in young rats ranged from two to ten parasites per cell and occasionally up to fifteen parasites were counted in one cell. In older rats, the number of polychromatophilic cells in the circulating blood was markedly reduced (Fig. 1(C) and 1(D)). The stimulus for the production of polychromatophilic cells has not been determined. The fact that a higher percentage was produced in the circulating blood of young rats suggests that the phenomenon is a host response and not induced directly by the malaria parasite. A comparison of the course of infection in young versus old rats suggests that the polychromatophilic response is the basis for the development of high parasitemia and hence greater pathogenicity and higher mortality (Fig. 1).

THE EFFECTS OF *Haemobartonella muris* ON *P. berghei*

Early in this study, the observation was made that *P. berghei* was inducing the appearance of *H. muris* in the circulating blood. There appeared to be a correlation between the severity of *P. berghei* infection and the degree of *H. muris* infection. In rat No. 81, after the appearance of *H. muris* in the blood stream, the erythrocyte count fell rapidly within two to three days from 6,360,000 to 1,720,000 and, finally, to 800,000, which is a lower count than has been observed with either

infection alone. The acute blood stream infection with these two parasites caused the red cells to increase in size, and the consistency of the blood became watery and of diminished viscosity. Sometimes, instantaneous coagulation occurred, precluding the making of blood films. The blood also appeared to be hemolyzed.

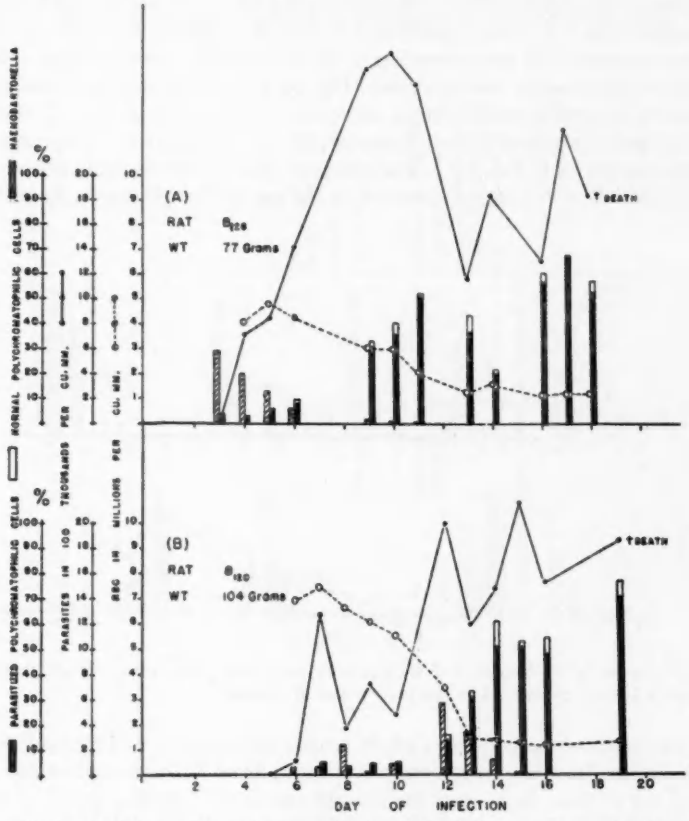

FIG. 2. Effects of *H. muris* on the parasitemia of *P. berghei* and the production of polychromatophilic cells.

In rats weighing less than 100 grams, *H. muris* appeared between the third and seventh day after inoculation with *P. berghei* (Fig. 2(A)). In older rats of 100 to 150 grams, hemobartonellosis appeared from the seventh to the twenty-first day with only a light infection developing (Fig. 2(B)). In older rats weighing up to 300 grams, hemobartonellosis remained latent after the development of *P. berghei* infection; but, after splenectomy, infection with *H. muris* became intense (Fig. 3(A)). In the younger rats, infection with *H. muris* varied in intensity, ranging

from 10 to 80 per cent of erythrocytes parasitized, and persisted until death occurred. In a small percentage of rats, *H. muris* could be detected for a few days before the infection disappeared to become latent. However, in all cases, *H. muris* disappeared before the development of acute anemia (Fig. 2), as was indicated by Tyzzer (1942).

The most remarkable effect of the developing acute anemia caused by hemobartonellosis was a sudden increase in numbers of *P. berghei* followed by a drop and then another rapid rise, exceeding the previous peak. Consequently, a zigzag intensity of parasitemia was produced (Fig. 2). During the course of associated infection of *P. berghei* and *H. muris*, there was a progressive increase in the production of polychromatophilic erythrocytes, but in certain animals the production fluctuated as shown in Fig. 2(A). The effects of splenectomy in rats infected with *P. berghei* and *H. muris* were dependent on the age of the rat, time lapse between

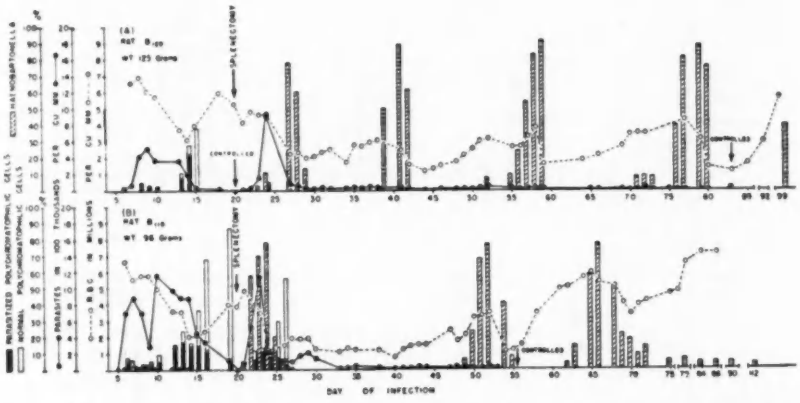

FIG. 3. Course of *P. berghei* and *H. muris* in rats after splenectomy. Parasitemia of *P. berghei* is greatly prolonged in the presence of *H. muris*.

the development of parasitemia with *P. berghei* and surgery, and intensity of *H. muris* infection. In rats spontaneously recovering from *P. berghei* infection, splenectomy ten or more days after the disappearance of *P. berghei* from the blood did not induce the recurrence of this malaria parasite, but it did initiate the appearance of hemobartonellosis (Fig. 3(A)). However, rats spontaneously recovering from the malarial infection, as determined by repeated thin- and thick-film examinations, showed relapse when splenectomized within two to five days after the disappearance of *P. berghei* from the blood stream. In two rats this malarial infection remained patent for fifty-four and eighty days after the first inoculation of *P. berghei*. Furthermore, *H. muris* appeared after splenectomy, also producing a severe infection that relapsed several times (Fig. 3). In these animals, hemoconcentration developed when the animals refused water and food, and they became moribund. Two additional rats, as shown in Figs. 3(A) and 3(B), still showed *H. muris* in red cells for more than one hundred days following splenectomy.

P. berghei IN *Haemobartonella*-FREE RATS

A total number of thirty-six rats have been used for this part of the study, eight from a Sprague-Dawley strain and the rest from Carworth Farms. Only five out of thirty-six rats died from the infection with *P. berghei*. With the exception of one which weighed 115 grams, all weighed less than 65 grams at the time of inoculation. The highest parasitemia in these rats was 2,524,440 parasites per cu.

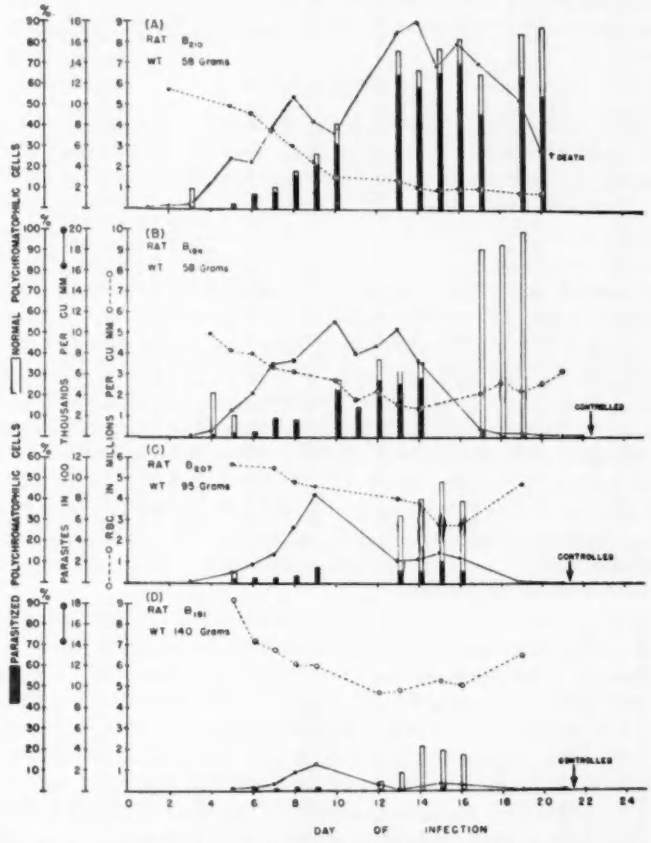

FIG. 4. Course of infection with *P. berghei* in *Haemobartonella*-free white rats.

mm., a lower rate than that of rats with the associated infections as given in Table 1. The death rate in these rats was 13.8 per cent, as compared with 50 per cent in rats with *H. muris*. Furthermore, the malarial infection was controlled in young rats, even in those which weighed less than 60 grams (Fig. 4(A)), while in rats with associated infections death generally occurred. In *Haemobartonella*-free rats with *P. berghei* infection, the production of the polychromatophilic cells

TABLE 2

Course of P. berghei *infection in* Haemobartonella*-free white rats*

RAT		INOCULUM DOSAGE/KG. AND ROUTE	INCUBATION PERIOD	PATENT PERIOD	HIGHEST PARASITEMIA OCCURRED			DEATH	CONTROLLED INFECTION
No.	Wt.				Day	Percentage	Parasites per cu. mm.		
	gm.		*days*	*days*				*days*	*days*
191 ♀	140	60 M.—t.v.	5	17	5	4.2	249,900		23
192 ♀	140	120 M.—t.v.	5	17	5	8.3	431,600		23
193 ♀	95	60 M.—t.v.	4	18	6	8.6	442,900		22
194	58	60 M.—t.v.	3	20	8	41.0	1,102,900		24
195	57	60 M.—t.v.	4	22	9	50.1	1,157,310		27
196	95	60 M.—t.v.	3	17	10	100.5	1,718,550		21
198 ♀	105	60 M.—t.v.	3	20	7	4.7	261,790		25
199 ♀	105	60 M.—t.v.	3	15	6	8.2	381,300		21
200 ♀	105	180 M.—t.v.	2	20	13	7.7	322,630		23
205 ♀	100	60 M.—t.v.	3	19	7	29.3	1,119,260		23
206	100	60 M.—t.v.	3	19	15	96.6	1,535,940		23
207 ♀	95	60 M.—t.v.	3	18	7	18.6	853,740		22
208 ♀	95	60 M.—t.v.	1	15	7	9.6	558,720		17
209	58	60 M.—t.v.	1	21	15	138.6	2,148,300		23*
210	58	60 M.—t.v.	1	20	14	157.2	1,823,520	22	
215 ♀	115	60 M.—t.v.	2	15	4	19.3	1,109,750		18
216	115	60 M.—t.v.	2	17	12	111.5	2,218,850	20	
217 ♀	145	60 M.—t.v.	3	14	4	3.9	238,680		18
219	240	90 M.—t.v.	4	16	7	8.2	426,400		21
220	240	60 M.—t.v.	2	21	13	35.1	972,270		24
221	43	60 M.—t.v.	1	9	9	60.2	1,089,620	11	
222	40	60 M.—t.v.	1	9	9	114.6	2,120,100	11	
223	65	60 M.—t.v.	2	13	12	138.6	1,663,200	17	
224	65	60 M.—t.v.	5	15	10	66.5	1,556,100		21
225	67	60 M.—t.v.	4	17	10	130.8	2,524,440		22
227	75	60 M.—t.v.	3	21	12	75.2	1,707,040		25
228	65	60 M.—t.v.	5	18	9	47.7	1,259,280		24

♀ = Female.

* Paludrine given on the 8th day.

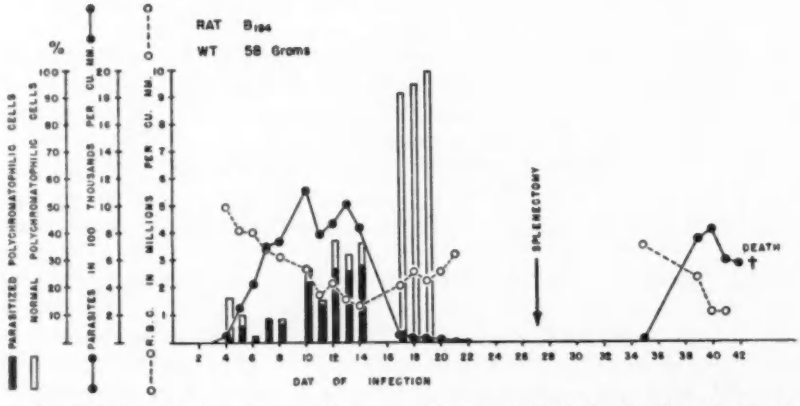

FIG. 5. Effect of splenectomy on the course of *P. berghei* infection in *Haemobartonella*-free white rats.

was lower (Fig. 4(C) and 4(D)), the average incubation period was shorter, the daily drop of red blood count was more gradual, and death took place at a higher red blood count, as shown in Table 2 and Fig. 4.

Five of the *Haemobartonella*-free rats were splenectomized between two to seven days after the infection of *P. berghei* was controlled. Six to twelve days after splenectomy, the infection of *P. berghei* relapsed in all of them. Four of these rats developed low parasitemia, and the infection remained patent for two to four weeks. However, one animal died on the fourteenth day after splenectomy with a peak parasitemia of 805,350 parasites per cu. mm. and red cell count of 1,050,000 (Fig. 5).

DISCUSSION

Studies on the course of infection with *P. berghei* in white mice, hamsters and white rats have shown that the infection in white rats can be controlled more effectively for experiments requiring quantitative data about the parasites and interpretation of the course of infection. The consistency of the results in white rats, however, depends upon inoculation of controlled numbers of parasites per kilogram of body weight. The wide differences of weight among rats coming from the same litter invalidated the use of age only as a criterion to determine the size of the inoculum of parasites. The results have shown that size of dosage affects the course of infection and that young white rats weighing 60–100 grams are more susceptible with a higher mortality rate than rats weighing 150–300 grams. This age difference in respect to susceptibility, course of infection and mortality has also been mentioned by Galliard and Lapierre (1951). This response, from the data, suggests a direct correlation between the hematopoietic response of polychromatophilic cells and the peak of parasitemia reached. *P. berghei* prefers the immature erythrocytes of white rats, a finding which was reported also by Galliard (1949) and Baldi (1950) in white mice. In our studies, parasites in mature erythrocytes seldom achieved normal size and number of merozoites per segmenter.

The finding that *P. berghei* infection in white rats caused the activation of latent *H. muris* infection and increased reticulocyte response leading to intense parasitemia of the plasmodia complicated the standardization of experimental procedures with this infection in rats. According to Perla and Marmorston-Gottesman (1932), latent infection with *H. muris* is common in white rats, occurring in animals of carrier stock between the fifth and seventh week. The infection remains latent; and it is difficult, if not impossible, to detect the presence of the organism in the circulating blood by direct examination or by staining procedures. Splenectomy or diseases which severely injure the splenic pulp may cause a flare-up of a latent infection with subsequent anemia. In 1927, Mayer, Borchardt, and Kikuth noted rare hemobartonellae in rats infected with *Trypanosoma lewisi*, but no anemia; and similar results were later reported by Taliaferro and other workers (Weinman, 1944). Splenectomy on rats with latent infections of *H. muris* is followed within six to ten days by a severe progressive anemia which is fatal in 20 to 25 per cent of rats (Perla and Marmorston-Gottesman, 1930).

The rats obtained from local dealers for use in the present study, with few exceptions, carried latent infections with *H. muris*. These parasites became visible

in red blood cells either after inoculation of *P. berghei* or after splenectomy. One of five normal rats splenectomized for controls developed acute hemobartonellosis with marked anemia and died on the third day. However, in a series of eight splenectomized rats with associated *P. berghei* and *H. muris*, severe relapses of the two infections with anemia occurred, but no animals died. Splenectomy markedly prolonged the patent period of both infections (Fig. 3), showing the synergism of these two associated infections and possibly reduced pathogenicity for the host. However, one of the five splenectomized *Haemobartonella*-free rats which weighed 58 grams at the time of inoculation died after splenectomy. According to Galliard and Lapierre (1950a), splenectomy in young rats of 50 grams followed by the inoculation of *P. berghei* three days later resulted in 100 per cent mortality, while in rats of heavier weight (100–250 grams) splenectomy did not seem to show any effect. Since the rats in our experiment were splenectomized after they had controlled their infections, they were of heavier weight, which fact might account for the low mortality rates in our splenectomized *Haemobartonella*-free rats.

In studies with mixed infections, seventeen female and sixty-three male rats were used. Thirteen female rats (ten weighing 70–95 grams and three weighing 145–165 grams) controlled their infections spontaneously, but only twenty-four male rats (one weighing 65 grams and twenty-three weighing 100 grams or over) controlled their infections spontaneously. In experimental infections with *Haemobartonella*-free rats, of which twenty-one were female and fifteen male, five male rats died. Weinman (1944) reported that both age and sex are factors affecting resistance in hemobartonellosis, the infection being more severe in the younger and in the male rats. Although the course of *P. berghei* infection in white rats is dependent on dosage and weight of the rat, the data suggest a sex difference also, but further experiments are needed to confirm this assumption statistically.

The synergistic effect of *H. muris* on *P. berghei* infection is the result of a preference by the *Plasmodium* for young polychromatophilic erythrocytes induced in greater numbers by hemobartonellosis. The activation of latent hemobartonellosis in the presence of infection with *P. berghei* is caused either by temporary blockage of the spleen resulting from antiplasmodial cellular activity or by the plasmodium-producing metabolic waste products needed by *H. muris* for growth and multiplication. With the rapid advances of our knowledge concerning synergism and antagonism of microorganisms (Smith and Martin, 1948), this interesting synergism of *Haemobartonella* and *Plasmodium* species in white rats provides a tool for studying the disease-producing activities of these parasites. This study suggests the interesting possibility that human malaria or other diseases activate latent infections of *Bartonella bacilliformis* in man that lead to the skin phase of Carrion's disease known as verruga peruana.

SUMMARY AND CONCLUSIONS

On the basis of available data in the present study of *P. berghei*, the following general conclusions seem to be justified:

1. Albino rats are the most satisfactory experimental hosts for high percentages

of parasites in intact red cells. The presence of extracellular *P. berghei* continuously in white mice makes quantitative studies difficult.

2. An inoculum of 60 million parasites per kilogram of body weight gives the most consistent incubation period and course of infection.

3. Differences in age affect the course of *P. berghei* in white rats. Younger rats under 100 grams develop higher parasitemia rates, while older rats either die with lower parasitemia rates or control infection by acquiring immunity.

4. *P. berghei* activates latent hemobartonellosis in white rats, causing intense infection with *H. muris*. The degree of polychromatophilia induced by *H. muris* reults in higher rates of parasitemia with *P. berghei*, with a peak of 191 per cent being recorded.

5. Infection with *P. berghei* in *Haemobartonella*-free rats reveals a shorter incubation period, a lower percentage of polychromatophilic erythrocytes, and the occurrence of death before acute anemia develops.

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RELATIONS BETWEEN THE ENDOHISTIOCYTIC FORMS OF *PLASMODIUM GALLINACEUM* AND THE IMMUNOLOGICAL COURSE OF THE INFECTION IN FOWLS INOCULATED WITH INFECTED BRAIN TISSUE*

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Jacobi (1939) and James (1939) noted that while in sporozoite induced infections of *Plasmodium gallinaceum* forms inside endothelial cells[1] are constantly found at the beginning of the patent infection, in blood induced infections they were found only in a certain number of fowls in the 3rd or 4th week from the inoculation.

The senior writer (1940, 1941a, 1942) observed that the type of the infection is different depending not only on the infective material used for inoculation, but also on the immunological course of the infection itself. In other words there are the following types of *P. gallinaceum* infections:

1. In fowls inoculated with sporozoites, or brain tissue with endohistiocytic forms, or infected blood of fowls having endohistiocytic forms in the internal organs, the patent infection always begins with the more or less simultaneous presence of both endoerythrocytic and endohistiocytic forms.

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[1] It will be noted that in this paper the word "exoerythrocytic" is never used, according to the views expressed by the senior author in several papers (Corradetti 1942, 1949; Corradetti *et al.* 1941).

The word "exoerythrocytic" was provisionally proposed by James and Tate (1938), when the nature of the host cell was not known and still under discussion. The word itself means "outside the erythrocytes", while the parasites are really "inside" other cells. It is well known today that different species of malaria parasites may develop inside: 1) red cells; 2) histiocytes and endothelia; 3) different types of blood and blood-forming cells; 4) hepatic epithelial cells.

All these cells are so different among themselves, histologically and embryologically, that it seems quite arbitrary, to give a special name only to forms developing inside red cells. A distinction made only between a) schizogonic forms which develop inside red cells and b) schizogonic forms which develop in cells other than red cells, does not have a scientific basis today. It is derived only from the fact that schizogonic forms were first found in red cells and only many years after in other cellular locations.

Many inconveniences arise from such an arbitrary distinction. For instance forms of *P. elongatum* developing inside basophilic erythroblasts and forms of *P. cynomolgi* developing inside epithelial cells of the liver are both considered "exoerythrocytic forms": it is evident that basophilic erythroblasts are very close histologically to red cells and very far, histologically and embryologically, from liver epithelial cells.

To avoid such confusions the senior author (1949) had already suggested that the following terms be employed: 1) *endoerythrocytic* forms for parasites multiplying in red cells; 2) *endohemoblastic* forms for parasites multiplying in hemopoietic cells; 3) *endohistiocytic* forms for parasites developing in cells of the reticulo-endothelial system, and 4) *hepatic endoepithelial* forms for parasites multiplying in epithelial cells of the liver.

2. In fowls inoculated with infected blood of fowls having no endohistiocytic forms in the internal organs, the patent infection always begins as purely endoerythrocytic. In the succeeding course of the infection endohistiocytic parasites either appear *suddenly at the 11th* day of blood positivity, or *never appear*, if the acquired immunity stops the primary endoerythrocytic attack by a parasitolytic crisis occurring before the 11th day. Similar results were obtained in their experiments by Villalobos (1940), Mosna (1940), De Ritis (1940), Mudrow (1940), Gramiccia and Saccà (1948), D'Alessandro, Oddo and Smiraglia (1948), and Verolini (1949).

The explanation of this behavior was given by the senior writer (1941a), who related the existence of a parasite development inside cells of the reticuloendothelial system to the absence or delay of production of an acquired immunity in the host.

In the last few years many workers have investigated *P. gallinaceum* infections produced by inoculation with different infective materials in various biological conditions. These researches are interesting because they resulted in the isolation of strains showing different biological properties. Haas and collaborators (1946, 1948) isolated two strains of different behavior: 1) Infections produced by alternating mosquito-chick passages in which endoerythrocytic parasites predominate (Pattern A); 2) Infections produced by blood transfer in which endohistiocytic forms predominate (Pattern C). Lewert (1948, 1950, 1950a), starting passages from forms developed in tissue culture, obtained in 9 subsequent passages of brain tissue through 7–32 day old chicks, infections in which only endohistiocytic parasites were detected. Greenberg, Trembley, and Coatney (1950) isolated two strains: 1) the BI strain unable and 2) the SP strain able to produce merozoites which could reproduce normally in the erythrocytes. In subsequent papers the same authors (1951, Trembley *et al.* 1951, 1951a) described many biological changes spontaneously or artificially produced in these strains.

This paper deals with the behavior of both endoerythrocytic and endohistiocytic forms of *P. gallinaceum* in relation to the immunological course of the infection (either spontaneous or modified by splenectomy and superinfection) in fowls inoculated with brain tissue containing endohistiocytic forms in the capillaries.

METHODS

The fowls of these experiments (3–6 months old) were inoculated in serial passages with an emulsion of brain tissue containing endohistiocytic forms in the capillaries. The passages were started from a blood inoculated fowl belonging to an uninterrupted series of 14 years of successive blood transfers. The fowl was sacrificed after 11 days of demonstrable infection in the blood and found positive for endohistiocytic forms. The brain of a fowl found positive for endohistiocytic forms was always employed for each subsequent passage. The inoculation was always made by the following method: the brain cortex was emulsified in physiological saline solution, and 1 cc. of the emulsion was injected into the pectoral muscles of the bird. The course of the infection in

each fowl was followed by daily blood examinations and by cerebral biopsies made at irregular intervals by the method of Verolini (1949) modified by Cavaceppi (1951).

EXPERIMENTAL

Experiment 1

In 19 serial passages 69 fowls were inoculated, and the results are summarized in Table 1. Of these 69 fowls, 67 became infected, all of which had normal endoerythrocytic primary attacks. It may be added that following the primary attack

TABLE 1

Results of serial inoculation of brain tissue containing endohistiocytic parasites

SERIAL NUMBER OF THE PASSAGE	NUMBER OF FOWLS INOCULATED IN EACH PASSAGE	NUMBER OF FOWLS IN WHICH PARASITES WERE DETECTED IN THE FIRST 25 DAYS		OBSERVATIONS
		Endoerythrocytic	Endohistiocytic	
1	4	4	3	
2	5	5	5	
3	8	8	2	Biopsy after the 20th day in 4 fowls
4	4	4	2	Biopsy after the 20th day in 2 fowls
5	4	4	4	
6	4	3	2	No biopsy in 2 fowls
7	6	6	4	No biopsy in 2 fowls
8	2	2	2	
9	3	2	2	No biopsy in 1 fowl
10	3	3	2	
11	3	3	3	
12	3	3	2	
13	2	2	2	
14	3	3	3	
15	3	3	1	No biopsy in 2 fowls
16	3	3	2	
17	3	3	3	
18	3	3	1	
19	3	3	1	No biopsy in 2 fowls

the blood was negative except for occasional findings of parasites in the red cells.

In 46 of 60 fowls examined, endohistiocytic forms were revealed. (In 6 of the negative ones the examination was probably too late, as explained by experiment 2). In each passage there were a number of fowls showing both endoerythrocytic and endohistiocytic parasites, which retained the faculty, through 19 passages, of invading both tissues.

Experiment 2

One hundred fifteen brain biopsies were made at different times on fowls inoculated in the preceding experiment. Some of the fowls were examined only

once, and some on the contrary were followed by frequent examinations of the brain tissue (as many as 8 in the same bird). The results (Table 2) show that endohistiocytic forms were found only between the 8th and the 25th day after inoculation. None of the subsequent 31 biopsies taken between the 26th and the 80th day was found to be positive for endohistiocytic forms. It may be added that the negative results between the 26th and the 80th day after inoculation were obtained mostly in fowls which showed positive results in biopsies taken between the 8th and the 25th day after inoculation. It appears that endohistiocytic forms disappear from the brain endothelium within 25 days after inoculation.

Experiment 3

Two fowls of the preceding experiments were reinoculated with brain tissue containing endohistiocytic forms on the 43rd and 84th day respectively after the first inoculation. Examination of the brain capillaries made on the 16th day

TABLE 2

Finding of endohistiocytic parasites by brain biopsies made at various intervals after inoculation

DAYS AFTER INOCULATION	TOTAL NUMBER OF BIOPSIES	POSITIVE	NEGATIVE
3rd– 7th	2	0	2
8th–12th	14	8	6
13th–17th	51	42	9
18th–25th	17	5	12
26th–35th	12	0	12
36th–45th	7	0	7
46th–55th	3	0	3
56th–65th	8	0	8
80th	1	0	1

after the second inoculation gave negative results, while two controls inoculated with the same material used for the second inoculation were both positive for endohistiocytic forms on the 16th day after inoculation. The normal course of the blood infection in the experimental fowls was not modified by the superinfection: both remained negative with occasional findings of blood parasites as in the normal course of infection, while both controls had normal primary endoerythrocytic attacks. Thus endohistiocytic localization could not be reproduced in fowls in which it had previously been demonstrated.

Experiment 4

Twelve fowls inoculated with brain tissue containing endohistiocytic forms were allowed to develop normal primary attacks with invasion of both endothelial and red cells. After the end of the primary attack there followed, as usual, a course of infection characterized by the disappearence of the endohistiocytic forms and negativity of the blood, sometimes interrupted by occasional findings of parasites in the red cells. These fowls were then splenectomized at various

times from the 31st to the 85th day after inoculation, and the subsequent course of the infection was studied by daily blood examinations and one or two cerebral biopsies. Splenectomy produced relapses, with high percentages of parasites in the blood, in 11 out of 12 fowls; the twelfth showed no modification of the normal course. In contrast, all the brain biopsies (16 in number) taken from the 4th to the 18th day after splenectomy were found to be negative for endohistiocytic parasites. It is to be noted that 9 of these fowls were all found negative for endohistiocytic parasites even after 11 days of continuous persistence of endoerythrocytic parasites in the peripheral blood. Thus relapses after splenectomy failed to produce endohistiocytic forms in the brain.

Experiment 5

Six fowls from experiment 4 were reinoculated 15 to 21 days after splenectomy with an emulsion of brain tissue containing endohistiocytic forms. The reinoculation of endohistiocytic forms failed to produce a new invasion of the endothelial cells by the parasites, for the 6 cerebral biopsies made 13 to 19 days after the reinoculation were negative. Endohistiocytic forms were found on the other hand in four control fowls inoculated with the same emulsion of brain tissue: these latter fowls also had a normal endoerythrocytic primary attack.

SUMMARY AND CONCLUSIONS

1. The faculty of *P. gallinaceum* for invading both endothelial and red cells was continuously maintained for at least 19 passages in serial infections of fowls obtained by the inoculation of brain tissue containing endohistiocytic forms.

2. Endohistiocytic parasites in the brain endothelium of fowls so inoculated are observable only transitorially between the 8th and the 25th day after inoculation, with the highest percentage of positive results between the 13th and the 17th. No reappearance of endohistiocytic forms occurred in the following unmodified course of the infection (observations from the 26th day to the 80th).

3. The reinoculation of endohistiocytic forms in fowls which previously had had an endohistiocytic localization did not produce it again.

4. Splenectomy produces relapses in the blood, but does not produce the reappearance of endohistiocytic forms. Endohistiocytic forms are not produced even in the case of continuous persistence of the parasites in the blood for more than 11 days.

5. Reinoculation of endohistiocytic forms after splenectomy does not cause the reappearance of endohistiocytic parasites in the brain capillaries.

6. The conclusion is justified that an immunological process stops the first invasion of the endothelial system within 25 days after inoculation. Subsequently the endothelial cells become resistant to new invasions by parasites; neither splenectomy, nor a new inoculation of endohistiocytic forms, before or after splenectomy, can bring about a new endohistiocytic invasion. After the 25th day the infection is confined to the forms located inside red cells, and these latter only show active multiplication when a lower immunity level is produced by splenectomy.

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AN ANTIMALARIAL ALKALOID FROM *HYDRANGEA*

XIII. The Effects of Various Synthetic Quinazolones Against *Plasmodium lophurae* in Ducks

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The antimalarial properties of crude extracts of plants belonging to the genera *Dichroa* and *Hydrangea* have been described in previous papers in this series (Duggar, in press; Ablondi *et al.* 1952; Hutchings *et al.* 1952). After the active principle was isolated in crystalline form from the leaves of a variety of *Hydrangea* sp. (Ablondi *et al.* 1952), degradation (Hutchings *et al.* 1952) and synthesis (Baker *et al.* 1952) proved it to be an alkaloid, 3-[β-keto-γ-(3-hydroxy-2-piperidyl)propyl]-4-quinazolone. The naturally occurring alkaloid was found to have a quinine equivalent of approximately 100 when tested in ducks infected with *Plasmodium lophurae*, and the dl form of the alkaloid prepared synthetically was about ½ as effective. Previous studies of alkaloids isolated from *Dichroa febrifuga*, and tested against *P. gallinaceum* in chickens (Coatney *et al.* 1950), *P. lophurae* in ducks (Henderson *et al.* 1949), *P. relictum* in canaries (idem), and *P. cynomolgi* in monkeys (idem), have given quinine equivalents of from 64 to 148. Among the names given to the naturally occurring alkaloids of *Dichroa febrifuga* have been Dichroine (Chou *et al.* 1947), γ-Dichroine (Henderson *et al.* 1949; Chou *et al.* 1948), and Febrifugine (Henderson *et al.* 1949). Febrifugine has been reported to be active against the Chesson strain of *P. vivax* in humans (Coatney *et al.* 1950). It has been demonstrated that Febrifugine and the naturally occurring antimalarial alkaloid isolated from *Hydrangea* sp. in these laboratories are identical (Ablondi *et al.* 1952).

Since the alkaloid from *Hydrangea* possessed a high quinine equivalent, and a method of synthesis was accomplished, it was considered desirable to investigate related quinazolones for antimalarial activity. The results of assays of 54 synthetic quinazolones against *P. lophurae* in ducks are described herein, and further data will be presented as the series is expanded.

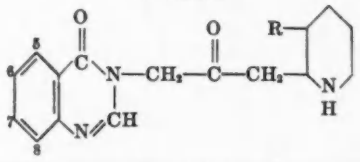
METHODS

White Pekin ducklings, 2 weeks old and about 200 grams in weight were used as experimental hosts. Infections with *P. lophurae* were produced by injecting intravenously 0.5 cc. of citrated blood from a heavily parasitized donor bird. The peak of parasitemia was reached on the 5th day in control birds, and blood smears were taken at that time to determine the effects of treatment. All of the compounds listed in the tables were soluble in water, and were usually supplied in the form of the hydrochloride salt. They were administered orally by tube twice daily for 3½ days. One dose was given on the day of inoculation, with two doses daily thereafter for 3 days. The dosage in milligrams per kilogram

which suppressed parasitemia below 10 per cent of the controls is listed in the tables as the suppressive dose.

The standard of comparison of activity given in the tables is the *Hydrangea* alkaloid equivalent. Since the alkaloid is approximately 100 times more effective than quinine against *P. lophurae*, quinine equivalents can be determined by multiplying the *Hydrangea* alkaloid equivalents by 100. The equivalents are in terms of the hydrochloride salts. When a sufficient quantity of the compounds was available, acute toxicity studies were made in mice. Mice weighing approximately 20 grams were treated orally twice daily for 3½ days, and the LD_{50} was recorded as the dosage which killed more than ½ of the mice within

TABLE 1



COMPOUND NO.	SUBSTITUTION					SUPPRESSIVE DOSE*	*Hydrangea* ALKALOID EQUIVALENT	C. I. INDEX	
	R=	5=	6=	7=	8=			Duck	Mouse / Duck
						mg./kg.			
794L	H					20–25	0.01	1	—
1697L	OH					0.1–0.2	1.0	4–6	5
1651L	CH_3O					2.0	0.1	4	—
1652L	CH_3O		CL			4.0	0.05	1	—
1656L	CH_3O		CH_3O			2.0	0.1	2	—
1657L	CH_3O		CH_3			4.0	0.05	2.5	—
1682L	AcO					0.3	0.7	6–7	—
U4074	OH[dl]					0.4	0.5	5	—

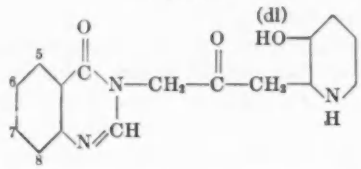
* Given orally twice daily for 7 doses, beginning on day of inoculation with parasites.

1 week. Similarly, the LD_{50} was determined in ducks with most of the compounds, although it is recognized that these figures are probably much less reliable than data obtained from mice. The chemotherapeutic index was determined by dividing the LD_{50} either in mice or ducks by the minimum dosage required to suppress parasitemia below 10 per cent of the controls. When no chemotherapeutic index is listed in the tables, the quantity of the compound available was not sufficient to arrive at this figure.

RESULTS

Data given in the accompanying tables are for the most part self-explanatory. The compounds listed in table 1 include the naturally occurring *Hydrangea* alkaloid (1697L), and the dl form of the alkaloid prepared synthetically (U4074). The remaining 6 compounds have hydrogen, methoxy or acetoxy groups sub-

TABLE 2



NO.	SUBSTITUTIONS				SUPPRESSIVE DOSE[a]	*Hydrangea* ALKALOID EQUIVALENT	C. I. INDEX	
	5=	6=	7=	8=			Duck	Mouse / Duck
					mg./kg.			
1698L	CL				0.3	0.7	7	10–11
1699L	CH_3				0.3	0.7	7	—
1736L	Br				0.4	0.5	8	12
1801L	CH_3O				0.6	0.3	3	10
1825L	CF_3				0.6	0.3	>10	10
1837L	NO_2				1.2	0.15	>3	6
1849L	I				7.5	0.03	>1	<1
1857L	$NHCOCH_3$				2.4	0.08	>4	>3
1684L		CL			0.2	0.1	2	—
1685L		CH_3			0.2	1.0	5	—
1688L		CH_3O			1.0	0.2	2	—
1753L		NH_2			>3.2	<0.06	—	—
1793L		Br			0.2	1.0	2	2
1800L		C_6H_5			>0.2	—	<1	<1
1822L		COOH			>3.2	<0.06	—	—
1694L			CL		0.4	0.5	5	—
1695L			CH_3		1.0	0.2	4	2
1727L			CH_3O		1.6	0.12	>2	—
1744L			Br		1.2	0.15	>2	3
1754L			NH_2		>3.2	<0.06	<1	—
1833L			CO_2CH_3		>3.2	<0.06	—	—
1836L			C_6H_5		>3.2	<0.06	—	—
1686L				CL	0.4	0.5	5	—
1692L				CH_3	1.0	0.2	5	—
1728L	CL	CH_3O			3.2	0.06	>1	1
1765L	CL	CH_3			0.3	0.7	5	10–11
1774L	CH_3	CL			0.6	0.3	1–2	2
1788L	CL	CL			0.3	0.7	3	5
1832L	CH_3	CH_3			0.3	0.7	3	10–11
1735L	BR		Br		1.6	0.1	>2	6
1762L	CH_3		CH_3		1.2	0.15	>3	3
1691L	CL			CH_3O	5.0	0.025	—	—
1726L	CL			CL	3.2	0.06	>1	2
1768L	CH_3			CH_3	4.0	0.05	—	>6
1794L	CL			CH_3	6.4	0.03	>1	>1

TABLE 2—*Continued*

NO.	SUBSTITUTIONS				SUPPRESSIVE DOSE*	*Hydrangea* ALKALOID EQUIVALENT	C. I. INDEX	
	5=	6=	7=	8=			Duck	Mouse / Duck
					mg./kg.			
1708L		CL	CL		0.1	1–2	2	2
1718L		CH_3O	CL		0.6	0.3	<2	6
1764L		CH_3	CL		0.8	0.25	2	2
1789L		CL	CH_3		0.3	0.7	2–3	2–3
1821L		Benzo			1.2	0.15	>3	10
1824L		CH_3	CH_3		0.3	0.7	5	5
1704L		CL		CL	0.2	1.0	1.5	1.5
1770L		CH_3		CH_3	0.6	0.3	3	1.2
1787L		CH_3		CL	0.6	0.3	3	5
1771L		CL		CH_3	1.2	0.15	1–2	1–2
1761L			CL	CH_3	2.4	0.08	>1	5
1767L			CH_3	CH_3	4.0	0.05	—	5

* Given orally twice daily for 7 doses, beginning on the day of inoculation with parasites.

stituted for the hydroxy on the piperidine ring, with or without substitutions in position 6 of the quinazolone nucleus. Several different batches of the natural alkaloid were assayed, and the suppressive dose varied between 0.1 and 0.2 mg./kg. The equivalents of the compounds listed in the tables were determined on the basis of 0.2 mg./kg. activity of the natural alkaloid. With the exception of the acetyl derivative and the synthetic dl form of the alkaloid, the remaining compounds listed in table 1 were much less active than the natural alkaloid, and gave lower chemotherapeutic indices in ducks.

In table 2, all substitutions were made on the 5, 6, 7 or 8 positions of the quinazolone ring. Fifteen compounds had equivalents of from 0.5 to 1.0, and 6 had chemotherapeutic indices (mouse/duck) of at least twice that of the naturally occurring alkaloid. Comparatively wide variations occurred within different groups of substitutions, both as regards the dosage required to suppress parasitemia and the chemotherapeutic index.

DISCUSSION

Most of the synthetic quinazolones related to the naturally occurring *Hydrangea* alkaloid have given some degree of activity against *P. lophurae* in ducks, and about 30 per cent have been at least ½ as active as the natural alkaloid (quinine equivalents of from 50 to 100). It is encouraging that slight changes in structure have varied both activity and the chemotherapeutic index. Substitutions in the 5 positions of the quinazolone ring have given the best ratios thus far between activity and toxicity, but several di-substituted compounds also appear promising. The effective dosage of the most active members of the group against *P. lophurae* compares favorably with the more potent 8-amino-quinolines. No effects have been observed with any of the quinazolones

listed against *Trypanosoma equiperdum* in mice, *Leishmania donovanii* in hamsters and *Schistosoma mansoni* in mice. Possible effects produced against the exoerythrocytic stages of avian malaria have not been investigated as yet.

SUMMARY

Data are given regarding the antimalarial effects against *Plasmodium lophurae* of a naturally occurring alkaloid isolated from *Hydrangea* sp., and 54 synthetic quinazolones related to the alkaloid.

Sixteen of the synthetic quinazolones were as effective or nearly as effective against *Plasmodium lophurae* in ducks as the naturally occurring *Hydrangea* alkaloid. Seven had chemotherapeutic indices (mouse/duck) of at least twice that of the naturally occurring alkaloid. The naturally occurring alkaloid was found to be approximately 100 times more effective than quinine against *Plasmodium lophurae* in ducks.

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THE COURSE OF NATURAL AND INDUCED INFECTIONS OF *PLASMODIUM FLORIDENSE* THOMPSON AND HUFF IN *SCELOPORUS UNDULATUS UNDULATUS* (LATREILLE)

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The observations reported here were made during a study of the infectivity of various sylvan malaria parasites to *Anopheles* mosquitoes. Both natural and induced infections of *Plasmodium floridense* Thompson and Huff (1944) were followed in the fence lizard *Sceloporus undulatus undulatus* (Latreille). Several aspects of *P. floridense* infections were observed that have not been reported previously. Since study of malarias in poikilothermic animals offers special advantages for investigating the effect of various external factors on the development of parasites, it is likely that saurian malaria parasites will be used in such investigations. The data presented here are intended to add information to the general characterization of these infections.

MATERIAL AND METHODS

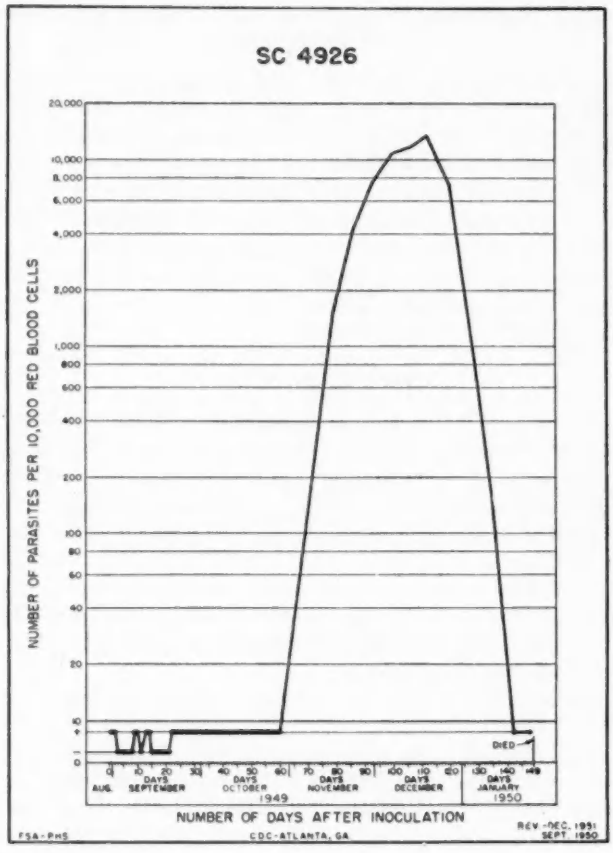
Infected lizards used in this study were collected in Baker and Early Counties in southwestern Georgia. Methods of maintaining lizards and techniques of laboratory manipulation were essentially the same as those employed by Thompson and Huff (1944). Lizards were kept in one gallon capacity glass jars provided with screened screw caps. The jars were kept on a stand with 100-watt light bulbs approximately one foot away. Lights were kept on for about eight hours each day. During this period, temperature inside the jar reached 32°C.; at other times, temperature inside the jar approximated room temperature, 25° to 35°C. Lizards were watered at least twice each week and were fed two or more *Tenebrio molitor* larvae twice weekly.

In enumerating parasites efforts were made to keep the probable error under 20 per cent. This was often not possible since only a very small quantity of blood could be obtained from some animals. The distribution of parasitized cells was apparently much less uniform in these cases than when the amount of blood was sufficient for preparation of a satisfactory film. Even if enough cells could be counted to permit a statistically valid density determination subsequent check counts often did not approximate the original figure; only when the same fields on the film were reexamined were comparable results obtained. The extremely high counts particularly are likely to be in error. Accordingly, the figures given for parasite densities must be evaluated with reservations. The measurements are believed to indicate trends reliably.

OBSERVATIONS AND DISCUSSION

Induced infections of *P. floridense* have been studied in several species of lizards, including *Sceloporus undulatus undulatus*, by Thompson and Huff (1944)

and Thompson (1944). They reported the following general characteristics of induced *P. floridense* infections in *S. u. undulatus*: the incubation period varies from less than a week to two weeks; the period of acute rise lasts 60 to 70 days and is terminated by a definite and rather sharp peak; parasitemia lasts about three months. The present report generally confirms these observations and

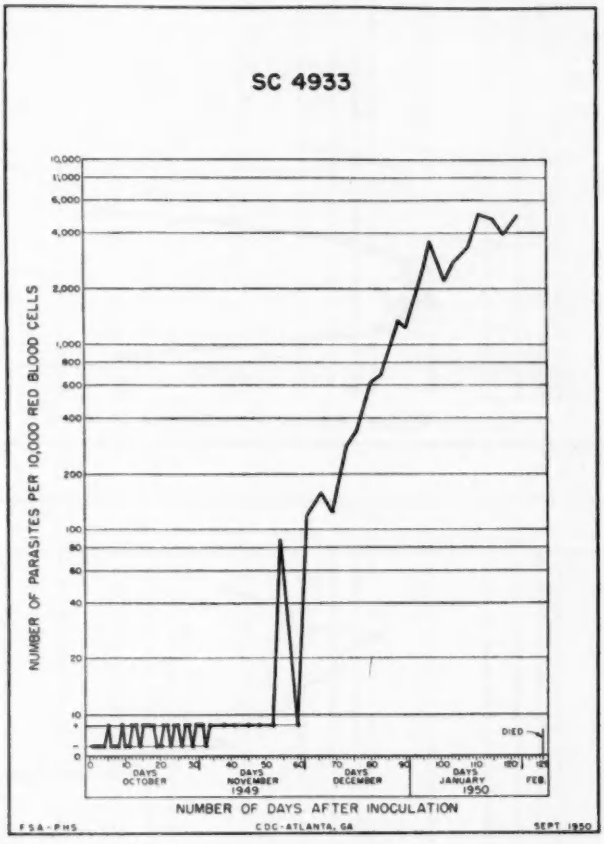


presents additional information on (1) factors influencing the initial rise of parasitemia, and (2) the course of naturally-acquired infections.

Observations of induced infections

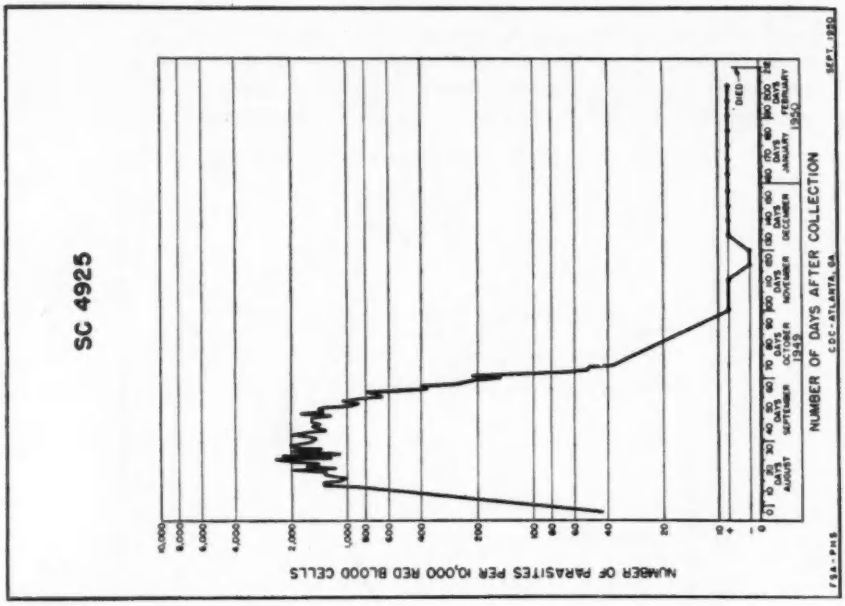
Considerable difficulty was experienced at first in obtaining results similar to those reported by Thompson and Huff. Following intracardial inoculation with more than 10,000 parasites, it was often possible to observe parasites in peripheral-blood films within a day or two. The density of parasites was so low,

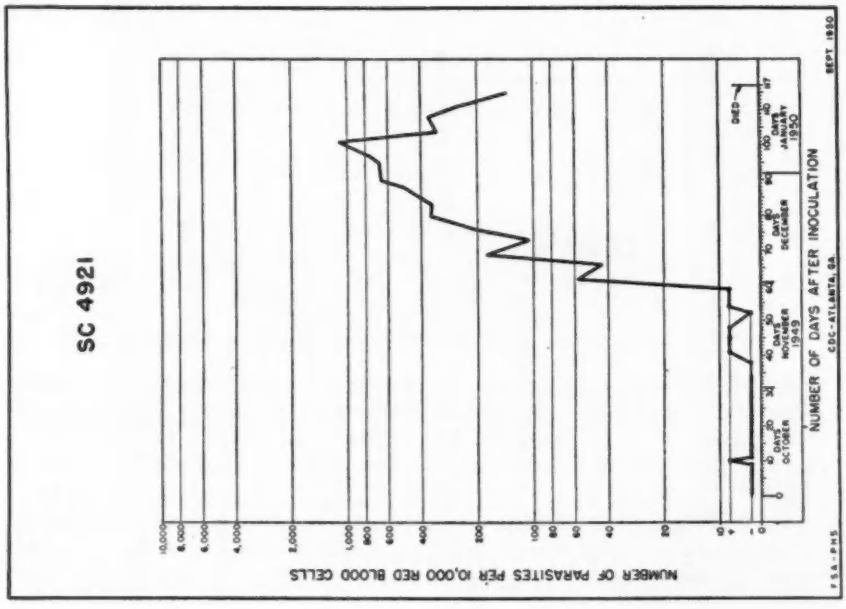
however, that statistically reliable measurements could not be made. When blood films were collected daily the inoculated animals usually died before the initial increase in parasites, although most lived for 30 days or longer following inoculation. Similar results were obtained until the frequency of blood sampling was decreased. In no instance was there a detectable increase in parasites when

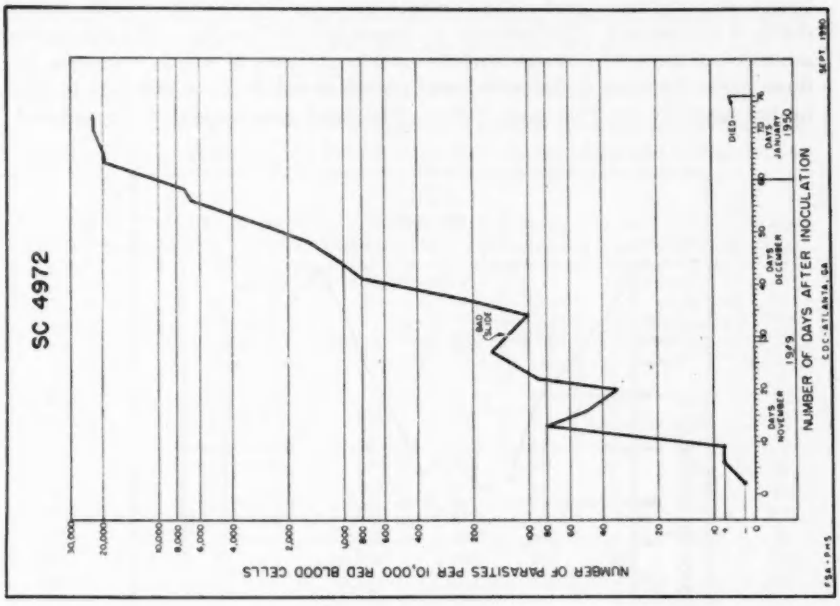


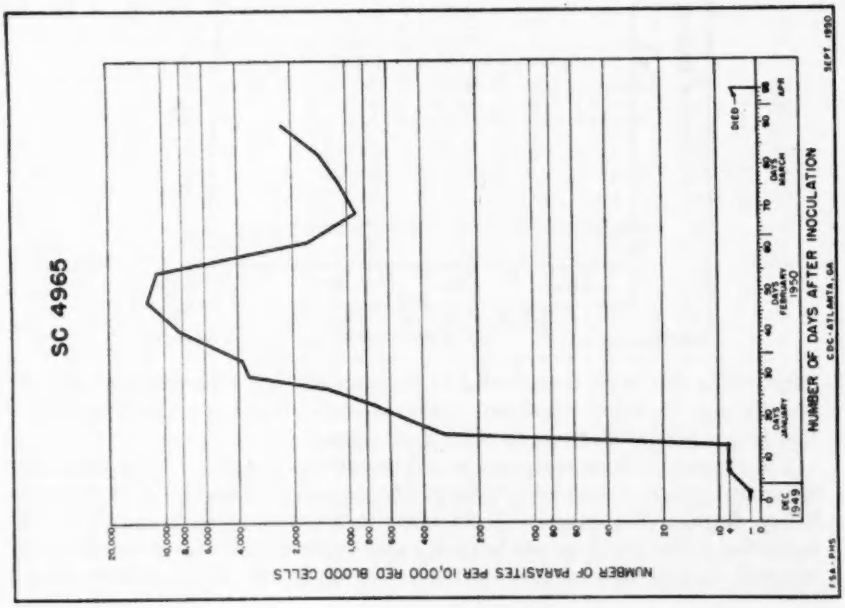
blood films were collected daily. A progressive rise was observed only when blood was obtained at intervals of at least a week. The course of infections of this type are shown for lizards SC 4926, SC 4933, and SC 4921. In each instance a pronounced rise in parasites began when daily examinations were discontinued.

Study of blood films collected at more frequent intervals indicated that weekly examinations were sufficient to provide reliable information on the course of infections. Bleeding at shorter intervals, as in SC 4925, appreciably reduced the

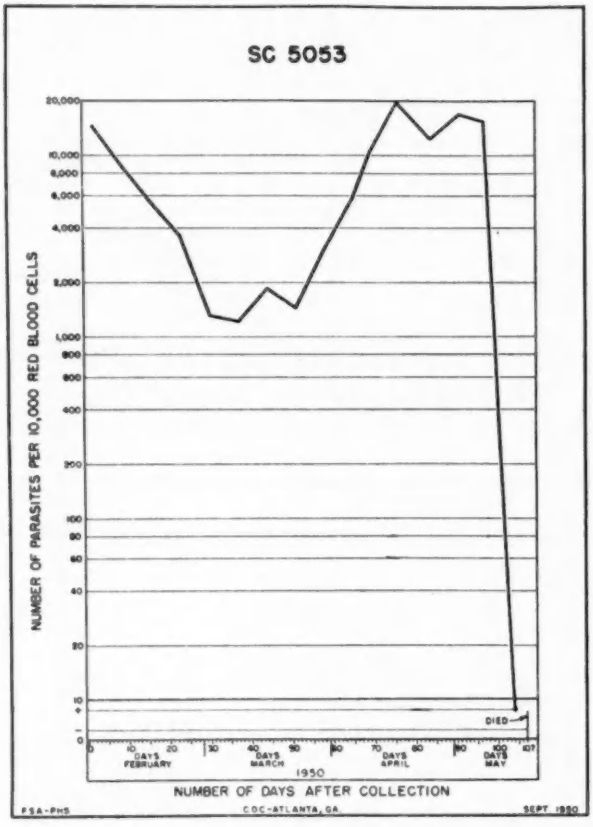

SC 4925
NUMBER OF PARASITES PER 10,000 RED BLOOD CELLS
NUMBER OF DAYS AFTER COLLECTION
CDC-ATLANTA, GA.
DIED
SEPT. 1950


SC 4921
NUMBER OF PARASITES PER 10,000 RED BLOOD CELLS
NUMBER OF DAYS AFTER INOCULATION
CDC-ATLANTA, GA.
DIED
SEPT. 1950


SC 4972
NUMBER OF PARASITES PER 10,000 RED BLOOD CELLS
NUMBER OF DAYS AFTER INOCULATION
CDC-ATLANTA, GA
SEPT. 1950
DIED
DAYS NOVEMBER 1949
DAYS DECEMBER
DAYS JANUARY 1950
BAD SMEAR


SC 4965
NUMBER OF PARASITES PER 10,000 RED BLOOD CELLS
NUMBER OF DAYS AFTER INOCULATION
CDC-ATLANTA, GA
SEPT. 1950
DIED
DEC 1949
DAYS JANUARY
DAYS FEBRUARY 1950
DAYS MARCH
APR

height of parasitemia and if maintained would probably have caused earlier death of the animals. The infection in lizards SC 4965 and SC 4972 are representative of the initial course when blood was obtained at weekly intervals. In these cases the incubation period and period of acute rise conformed to the results reported by Thompson (1944). The fatal termination of the induced



infections in this study is attributed to the large number of parasites inoculated directly into the blood. Thompson (1944) indicated that the intensity of infection probably depends upon the size of inoculum.

The present observations provide little tenable information on the length of the patent period of induced infections. The infection in lizard SC 4926 declined before death of the animal but the sharp decrease of parasites just prior to expiration of the host may not be comparable to the decline of parasites in cases where the host survives for several days after the patent period. (Similar sharp

recessions in parasite density immediately preceding death of infected lizards were noted in naturally-infected animals, *e.g.*, SC 5053.)

Observations of natural infections

Studies of infections in animals with naturally acquired parasites indicated that they follow, generally, a course similar to induced infections. Of more than

TABLE 1

Summary of observations of natural P. floridense *infections in* S. u. undulatus *indicating approximate density of parasites at various stages of infection and length of various stages*

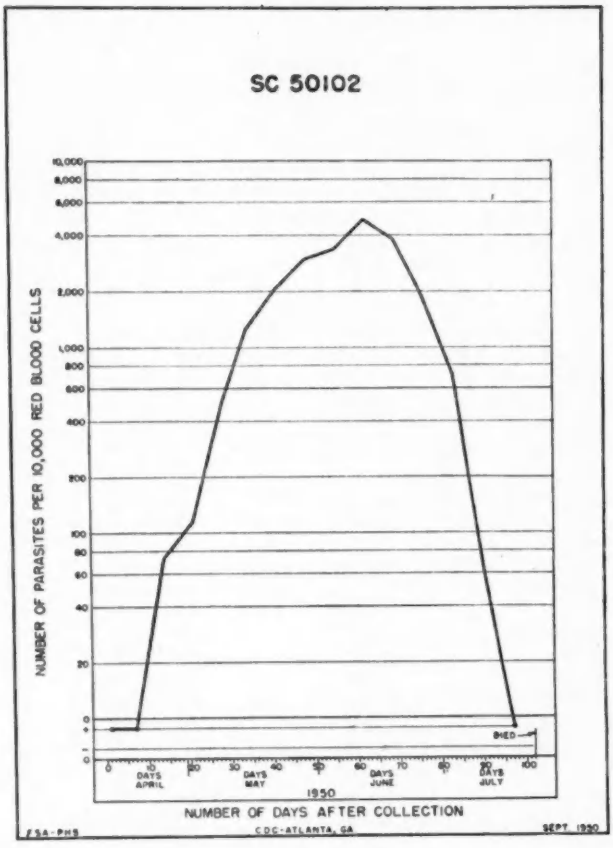
LIZARD NUMBER	DATE OF CAPTURE	DENSITY OF PARASITES/10,000 RBC			TIME IN DAYS			
		First examination	At peak	At last examination	Period of rise	Period of decline	Observed duration of infection	Total length of observations
4925	7/28/49	425	2,440	+	25	42	202+	202
4963	9/23/49	366	1,937	—	14	59	99	162
4990	10/17/49	675	2,537	588	37	84+	121+	121
4991	10/17/49	1,984	4,833	—	30	42	128	156
5015	1/25/50	648	6,194	+	27	46	87+	87
5019	1/25/50	2,590	13,636	3,666	42	†	292+	292
5032	1/31/50	—	625	613	64	†	53+	69
5033	1/31/50	+	12,500	3,406	76	42+	118+	118
5034	1/31/50	+	2,214	2,214	91	†	91+	91
5035	1/31/50	1,243	35,192	5,652	*	*	118+	118
5037	1/31/50	464	14,303	+	51	53	104+	104
5039	1/31/50	14,929	25,384	20,172	16	14	91+	91
5043	1/31/50	+	13,033	10,280	83	7+	90+	90
5053	1/31/50	14,577	19,843	+	*	*	104+	104
5057	1/31/50	777	14,143	1,435	44	53+	97+	97
5080	1/31/50	—	6,140	6,020	64	†	69+	69
5095	4/11/50	656	1,761	+	34	28	69+	69
5098	4/11/50	+	3,052	—	62	21	97	104
5099	4/11/50	91	7,145	—	48	35	97	377
50102	4/11/50	+	4,839	+	62	35	97+	97
50107	4/11/50	+	3,333	1,000	69	31+	90+	90
50183	8/1/50	+	327	+	42	63	210+	210
50209	8/9/50	140	412	+	†	†	110+	110
50210	8/9/50	+	200	+	89	14	131+	131
50224	8/28/50	+	451	+	84	28	143+	143
50240	10/10/50	—	6,474	—	50	20	70	132

* Infection irregular; see graph.

† Not observed.

50 infections studied, observations were made on 26 for more than two months. A summary of observations on these 26 specimens is given in Table 1. Since infections were acquired in nature prior to capture of the specimens, the period of rise was necessarily designated as the time from the first examination to the peak of parasitemia; in only three instances were the initial films obtained prior to development of patent infections. The period of decline could not be precisely

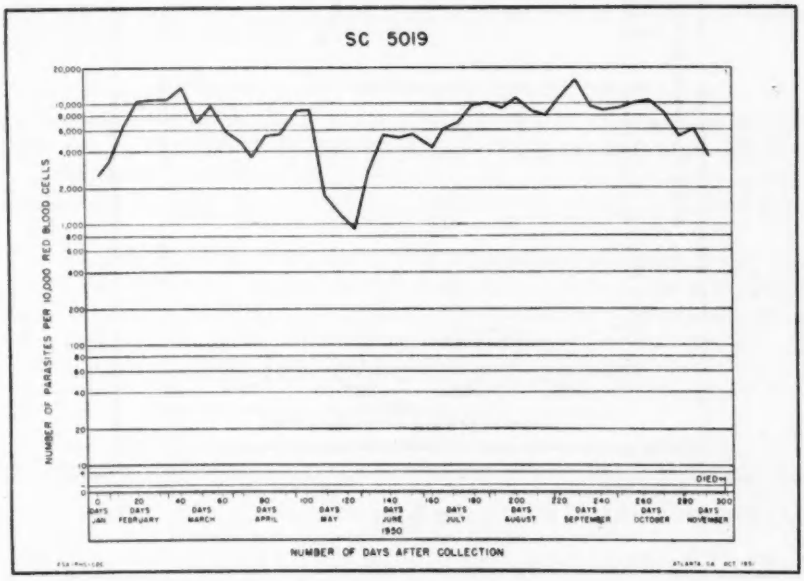
established because of the long persistence of parasites. The termination of the decline was designated as the point where parasite density was too low to permit an accurate count, probably less than 50 parasites per 10,000 red blood cells. End of the period of decline does not indicate absence of parasites. In cases where animals died before the parasite density declined to an unmeasurable



level, a plus mark follows the figure indicating duration of the period of decline. In only one instance (SC 50240) was the entire course of parasite development observed. Consequently, the figures for the observed duration of infections, in all but this case, are probably low. A plus mark following the figure for the observed duration of infection indicates that the infection was patent when the animal died.

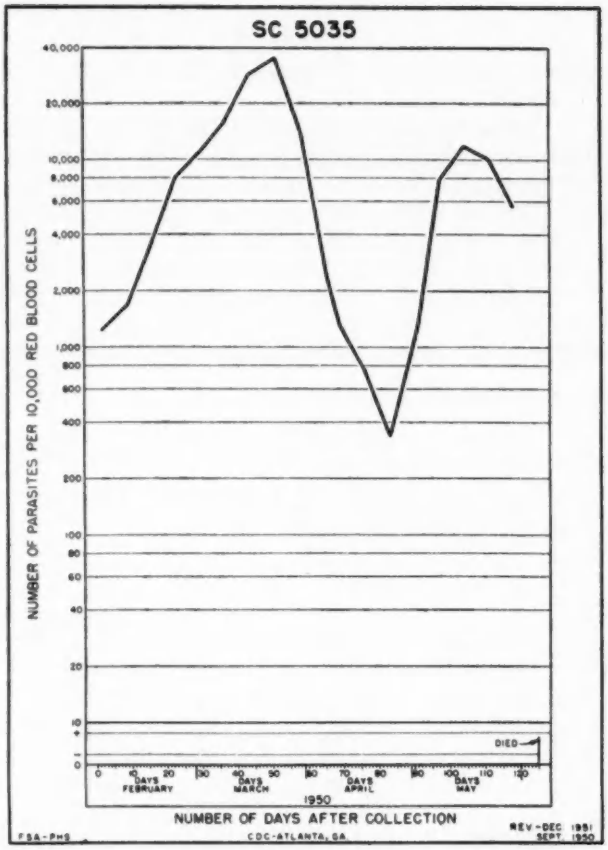
On initial examination, 12 specimens were found to have parasite densities too low for valid quantitative enumeration or were negative. Parasites subsequently

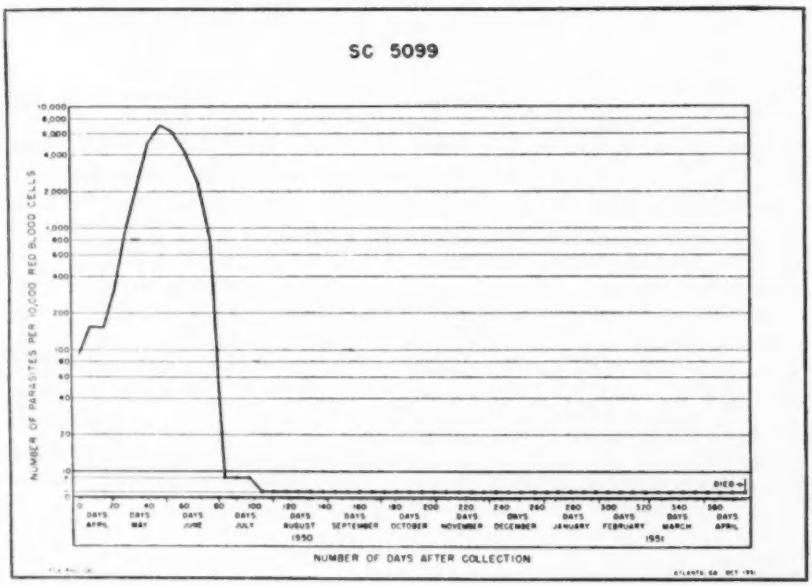
reached a peak in these lizards after a period of 42 to 91 days, average 70 days. It is likely that these infections were acquired in nature less than two weeks previously. As was reported (Goodwin, 1951) lizards maintained in the laboratory developed infections within two weeks if parasitemia became patent subsequent to capture. The only exception noted was specimen SC 50240 in which parasites were not demonstrable until 27 days after capture. Thirteen animals were observed where parasites reached maximum density and subsequently declined. The period of decline varies from 14 to 63 days, average 36 days. Thus, the average period of rise and decline, from these composite observations,



was approximately 120 days, allowing for 14 days incubation period. In consideration of data subsequently presented, it is assumed that all of the episodes observed were initial attacks. Both initial increase and decline were observed in six specimens. The initial attack in these cases lasted from 70 to 112 days, average 95 days. The graph for specimen SC 50102 shows a representative course of the initial attack.

Long duration of the patent period was an outstanding characteristic of these infections. Patent infections were observed for as long as 292 days in one instance (SC 5019), with parasite density varying between approximately 1,000 and 10,000 parasites per 10,000 erythrocytes during the period. Several other infections were also sustained at high levels for many weeks, *e.g.*, SC 5035. Several cases were observed in which parasites declined to such a low density that accurate parasite enumeration was not practical. In these cases, *e.g.*, SC


SC 5035
NUMBER OF PARASITES PER 10,000 RED BLOOD CELLS
40,000
20,000
10,000
8,000
6,000
4,000
2,000
1,000
800
600
400
200
100
80
60
40
20
10
+
1
0
DIED
0
10 DAYS FEBRUARY
20
30
40 DAYS MARCH
50
60
70 DAYS APRIL
80
90
100 DAYS MAY
110
120
1950
NUMBER OF DAYS AFTER COLLECTION
FSA-PHS
CDC-ATLANTA, GA
REV-DEC 1951
SEPT 1950


SC 5099
NUMBER OF PARASITES PER 10,000 RED BLOOD CELLS
10,000
8,000
6,000
4,000
2,000
1,000
800
600
400
200
100
80
60
40
20
10
+
1
0
DIED
0
20 DAYS APRIL
40 DAYS MAY
60 DAYS JUNE
80 DAYS JULY
100 DAYS AUGUST
120 DAYS SEPTEMBER
140 DAYS OCTOBER
160 DAYS NOVEMBER
180 DAYS DECEMBER
200 DAYS JANUARY
220 DAYS FEBRUARY
240 DAYS MARCH
260 DAYS APRIL
1950
1951
NUMBER OF DAYS AFTER COLLECTION

4925, infections remained patent but at very low levels for many weeks. In only five instances were infections observed to subside to a level where parasites could no longer be detected. In these cases there was no evidence of renewed parasite activity during the remaining observations. One infection (SC 5099) was observed for approximately nine months after end of the patent period without subsequent detection of parasites. The termination of these infections following an observed patent period of 70 to 128 days, average 98 days, is in contrast to the persistence of patent periods in other lizards, *e.g.*, SC 4925, SC 5019, SC 50183.

Although relapses were not observed, there were indications of diminution and increase of parasite production during period of patency in some of the lizards (SC 5035, SC 5053, SC 5019).

SUMMARY

The following observations resulted from studies of natural and induced infections of *Plasmodium floridense* infections in *Sceloporus undulatus undulatus*.

1. No demonstrable increase in parasite density occurred if blood films were made daily. Frequent blood collections apparently depressed the peak of parasitemia.

2. Massive inoculum caused early death of experimental animals and, in this study, inoculated animals died during or soon after the acute rise of parasites.

3. No relapses were observed in naturally infected animals; decline and increase of parasite production was observed in a few instances.

4. Long patent periods were characteristic of the infections.

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AN EPIDEMIC OF ACUTE ANTERIOR POLIOMYELITIS IN PANAMA IN 1950–1951

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In recent years attention has been focused on the occurrence of epidemics of acute anterior poliomyelitis in tropical countries where the disease previously was known to exist only in sporadic form or had escaped recognition entirely. Data concerning poliomyelitis as observed in the tropics has been ably reviewed by Rhodes (1948), Van Rooyen and Rhodes (1948) and Hammon (1949). Such epidemics have been characterized by a tendency to affect primarily infants, a type of incidence usually considered as indicative of a high degree of immunity in the older age groups due to repeated exposure to the etiologic agent, in spite of the low recorded incidence of the clinical disease. In the present report we wish to describe briefly an epidemic of acute anterior poliomyelitis which began in Panama, R.P. in August of 1950 and conformed to the pattern noted above. Three strains of the virus were isolated from suitable patients and studied.

Accurate statistical data concerning the prevalence of infantile paralysis in the Republic of Panama are not available. A total of 44 cases were reported to the Department of Health from 1943 to 1949, the accuracy of the diagnosis of many of which may be questioned. Annual distribution of cases varied between 2 and 6 with the exception of 1947 when 20 cases were reported. Hernandez (1948) noted the occurrence of an outbreak of some 20 additional cases in the Province of Chiriqui in the last months of 1946 and the beginning of 1947. It is thus obvious that 1947 was a year of increased prevalence. In the Canal Zone, Stevenson (1951) reported a total of 143 cases of local origin treated between 1903 and 1950. These figures indicate that clinical poliomyelitis has been present at a low endemic level in Panama for many years. However, the number of cases had never been sufficiently large to attract the attention of the medical profession in general or of the public. Many competent physicians and most of the lay population were unaware of the previous existence of the disease in the Republic and regarded its occurrence in 1950–1951 as entirely without precedent.

An effort was made to hospitalize all cases occurring in the Republic of Panama in the largest public hospital, Santo Tomas. From August 1950 to April 1951 a total of 133 patients with poliomyelitis were hospitalized in Panama. There were 9 deaths. During this epidemic period, Dr. G. M. Stevenson informs in a personal communication that 9 Panama residents were hospitalized in the Canal Zone, with one death. These figures give a total morbidity of 142 for the Republic or 18 per 100,000 and a mortality of 7 per cent. For the District of Panama where the great majority of the cases occurred, the morbidity rate was 51 per 100,000. We shall confine the remainder of our discussion to the 133 cases hospitalized in the Republic, for which we have more complete data.

The epidemic began in the poor and overcrowded district of Chorrillo in

the City of Panama, the first patient being a one year old infant, Arias, who died August 5, 1950, 12 hours after admission to the hospital and 3 days after the onset of illness with fever and catarrhal symptoms. Clinical, pathological and laboratory data all supported a diagnosis of acute anterior poliomyelitis and a strain of the virus was isolated from the spinal cord, thus providing the first incontrovertibly established diagnosis for the Republic. The second clinical case appeared in the same neighborhood in September. The number of cases then increased rapidly to reach a peak in January of 1951. In Panama the winter or rainy season extends from May to December and the summer or dry season from January until April. Thus, the epidemic began near the middle of the rainy season and reached a peak near the beginning of the dry season. A distribution of the cases according to the month of admission is presented below:

Month	*Year*	*No. cases*
August	1950	1
September	1950	1
October	1950	16
November	1950	27
December	1950	37
January	1951	40
February	1951	6
March	1951	3
April	1951	2

Age distribution showed greatest prevalence in children less than two years old, among whom 74 cases or 56 per cent of the total number were registered. Eighty-one per cent of all cases occurred in children less than 5 years old. The sex incidence was similar to that usually observed, there being a slight preponderance of males in a proportion of 69 or 51.9 per cent to 64 or 48.1 per cent. Data as to age distribution are presented below:

Age	*No. cases*	*Percentages*
1–11 months	20	15.0
12–23 months	54	40.6
2–5 years	33	24.9
6–10 years	13	9.8
11–15 years	6	4.5
19–39 years	7	5.3

The greater part of the cases, 91, were from the City of Panama and its suburbs. An additional 29 cases occurred in the Interior of the Republic within a radius of some 50 miles of the City, and the remaining 13 in the more distant provinces. Four of the cases occurring in the Interior had visited or resided in the City of Panama 7 to 12 days before developing symptoms.

Multiple family cases were observed in two instances. In one moderate-income urban family two sisters, aged 6 and 12 years respectively, both developed the paralytic form of the disease, the elder three weeks after the younger. In another almost destitute family living under extremely unsanitary conditions on the outskirts of the city three sisters aged 18 months, 32 months and 10 years

respectively were admitted to the hospital between the 24th and 27th of November, indicating a common source of contagion. The 10 year old girl showed the nonparalytic form of the disease, the other 2 the paralytic. The youngest sister is of especial interest as the only patient in this series who suffered a relapse, with flaccid paralysis of the legs on both hospitalizations. The relapse occurred 11 days after her dismissal as completely recovered on December 27. An attempt was made to isolate virus from her feces, collected on January 15, 1951. One *M. mulatta* was inoculated intraperitoneally and intranasally and six each of 2 day old white Swiss mice intraperitoneally without success.

In this series of 133 cases, 29 were classified as nonparalytic and 104 as paralytic, with 9 deaths all of bulbo-spinal type. Of the 95 nonfatal paralytic cases the following clinical classification was made:

Type of paralysis	*No. cases*
Quadriplegia and paralysis of the diaphragm	4
Quadriplegia	8
Paraplegia	57
Left inferior monoplegia	8
Right inferior monoplegia	6
Paraplegia and superior monoplegia	6
Right hemiplegia	2
Right superior monoplegia	1
Paralysis of the facial nerve	3

There were 24 patients with moderate to severe neuromuscular sequelae at the time of writing. Four of these show an apparently irreversible flaccid paraplegia, eight can walk with the aid of orthopedic appliances and 12 can use their impaired limb unaided. The prodromal symptoms followed more or less the classic pattern with fever and muscle pain present in all cases. Other symptoms observed in the order of their frequency were vomiting, catarrhal symptoms, diarrhea and headache.

With the exception of one case which was first seen in the late paralytic period, spinal fluid was obtained from all patients. Findings were typical of those usually observed. White cell counts varied from 15 to 1100, usually with a preponderance of lymphocytes. The average white cell count for all fluids examined was 60.

ISOLATION OF THE VIRUS

During the epidemic we isolated three strains of the virus of poliomyelitis, one from spinal cord and two from feces, all similar in animal pathogenicity. The first strain was obtained by the combined intracerebral, intraperitoneal and intranasal inoculation of one *Macaca mulatta* with a 10 per cent suspension of sections taken from various levels of the spinal cord of the first case, Arias, who died August 5, 1950, 12 hours after admission to the hospital. Six days after inoculation the animal developed fever followed in 3 days by the appearance of flaccid paralysis of the right leg which rapidly spread to involve also the left. At this time the animal was sacrificed. This strain has been maintained for a

total of 6 monkey passages. It proved infective for the Panama whiteface monkey, *Cebus capucinus imitator*, and two subspecies of the howler monkey, *Alouatta palliata palliata* and *A. palliata aequatorialis*, after two or more passages in the rhesus monkey. Experimental work on the susceptibility of the whiteface and howler monkeys has been reported elsewhere by one of the authors (Rodaniche, 1952). Suspensions of the original human cord and of monkey cord after each serial passage were injected intracerebrally into 6 infant and 6 adult white mice, 2 guinea pigs, 1 rabbit and 1 hamster with consistently negative results. Cotton rats became available after five monkey passages had been effected and two groups of 12 each were inoculated with fifth and sixth monkey passage virus respectively, also with negative results.

The other two strains were obtained from the feces of two patients with the paralytic form of the disease, Gordon, a 3 year old male and Diaz, a 2 year old male child, both admitted in October 1950. The feces were obtained 20 and 13 days respectively after the onset of symptoms. One *Macaca mulatta* was inoculated intraperitoneally with 10 cc. of an ether-treated 10 per cent fecal emulsion of each patient prepared according to the technique described by Paul (1948) and intranasally over a period of 5 days with 1 cc. in each nostril of the non-etherized fecal suspension. Prophylactic treatment against possible bacterial infection with sulfathiazole and penicillin was administered. Both animals developed febrile temperatures, tremors, muscular spasticity and extensive flaccid paralyses involving all four limbs within 11 days of the first injection. Attempts to pass these two strains to mice, guinea pigs, rabbits and hamsters gave negative results. The Gordon strain was given one further passage in the rhesus monkey. The Diaz strain was not passaged further. Sections of the spinal cords of affected monkeys showed the characteristic pathological picture with focal and perivascular infiltration with round cells, chromatolysis and necrosis of neurons and neuronophagia, most prominent in the anterior horns.

The epidemic here described was caused by a monkey-pathogenic strain of virus. If we employ the system of nomenclature recommended by the Committee on Nomenclature of the National Foundation for Infantile Paralysis (1948), the strains isolated here may be designated Arias-Panama-1950-M_6, Gordon-Panama-1950-M_2 and Diaz-Panama-1950-M_1. The Arias strain was sent to a laboratory in the United States for immunological typing. Results are still pending.

Attempts to isolate a Coxsackie virus from feces of 8 paralytic and 2 non-paralytic patients by the inoculation of 3–4 day old white Swiss mice gave negative results.

DISCUSSION

The epidemic of acute anterior poliomyelitis here described is of interest as it represents the first large outbreak in the history of the Republic, although a markedly increased incidence was observed in 1947. It began in the City of Panama where the great majority of the total number of cases were registered. We have no explanation to offer for this sudden change in the epidemiology of

the disease here. Sanitary conditions in the city are fairly good and have been more or less unchanging during the last 20 years except that during the war there was a great influx of people with resultant overcrowding which has continued up to the present time. As Panama is a crossroads of the world it is quite possible that an imported strain was responsible for the outbreak. However, as most of the adult population must be presumed immune since it escaped the clinical disease, such a strain would have to be antigenically similar to the local one or ones, though of increased virulence. As virus has not been isolated in Panama previously we have no basis for antigenic comparison of the present strain. We agree with Rhodes (1948) that "there is urgent need for further epidemiological and laboratory investigation in tropical countries" concerning poliomyelitis.

SUMMARY

The largest outbreak of acute anterior poliomyelitis which has occurred in the Republic of Panama is described. Infants less than 2 years old constituted a majority of the cases. Three strains of virus were isolated, one from cord and two from feces. All showed strict monkey pathogenicity.

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SURVEY OF LIVE FOREST ANIMALS FOR PROTECTIVE ANTIBODIES AGAINST YELLOW FEVER IN PANAMA, R. P.

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In November and December of 1948 a small outbreak of jungle yellow fever occurred near the town of Pacora in the Province of Panama, R. P. (Herrera *et al.* 1949; Courtney 1950). These cases represented the first to be identified here since 1907. In order to provide information concerning the vertebrate hosts of the disease in this area, a survey of live forest mammals for protective antibodies was initiated. Surveys of this kind have been conducted in Africa by Haddow *et al.* (1947), in Colombia by Bugher and coworkers (1944) and in Brazil by Laemmert *et al.* (1946) and Kumm and Laemmert (1950), but had not previously been undertaken in Panama. Later Clark (1950 and 1952) with the cooperation of the Yellow Fever Service, began a study of the immune status of shot arboreal forest animals in representative sections of the Republic. Although the survey of Clark was much more extensive and complete than the one here reported, it was considered of value to continue the study of live-caught animals as these present certain advantages over shot animals in the conduct of serological tests: *i.e.* the avoidance of nonspecific reactions due to contamination and hemolysis and the possibility of repeated bleedings to clarify unsatisfactory or inconclusive results.

MATERIALS AND METHODS

Animals were obtained principally by purchase. In most instances the animals were brought directly to the laboratory by local trappers. A few animals were bought in the market and their place of origin was unknown. This method of obtaining animals had the disadvantage that the majority were received from neighboring districts within easy transportation distance of the laboratory and relatively few from the more distant parts of the country. Blood was obtained either from the femoral vein or directly from the heart and the serum removed within 24 hours. Sera were stored in a deep-freeze compartment at approximately −20°F. until a sufficient number had been accumulated to test, a period never exceeding two months. Although Hughes and Perlowagora (1950) found that prolonged storage may alter the specificity of some nonprimate bloods, it does not affect sterile primate bloods. An effort was made to keep the animals alive for a period of at least two weeks after the first bleeding when they were retested for possible alterations in the neutralizing capacity of their sera. It was not possible to retest all the marmosets and night monkeys since many of these small and delicate animals died as a result of the initial bleeding or other causes.

The virus strain employed was the French neurotropic, received through the courtesy of Dr. Max Theiler of the Rockefeller Foundation. The standard intraperitoneal protection test in adult Swiss albino mice as devised by Sawyer and

Lloyd (1931) was employed as we considered it the most practical for a laboratory of limited facilities. Ten per cent virus suspensions were used and intraperitoneal inoculations made one hour after intracerebral injection of 2 per cent corn starch solution. The more recent tests of Bugher (1940) and Whitman (1943) are more sensitive in detecting minimal amounts of antibody. However, great sensitivity is at times disadvantageous in general survey work. As Smithburn (1951) says, "in the conduct of immunity surveys the purpose of which is to define the limit of the disease, it may be more important to exclude with certainty all nonimmunes than to determine the exact per cent of immunity. . . . In such cases it is unnecessary to employ a sensitively balanced test and it may be unwise to do so."

During the three year period 1949–1951, a total of 209 monkeys and 58 other animals were tested. For greater facility in presentation, data concerning pri-

TABLE 1

Results of the intraperitoneal protection test in Swiss mice for yellow fever with primate sera

SPECIES OF MONKEY	RESULTS OBTAINED				
	Positive	Negative	Inconclusive	Unsatisfactory	Total
Squirrel marmosets (*Marikina geoffroyi*)	5	74	3	3	85
Howler monkeys (*Alouatta palliata palliata* and *A. palliata aequatorialis*)	0	19	0	0	19
Whiteface monkeys (*Cebus capucinus capucinus* and *Cebus capucinus imitator*)	0	21	2	2	25
Red spider monkeys (*Ateles geoffroyi panamensis*)	4	20	0	0	24
Black spider monkeys (*Ateles fusciceps robustus*)	2	19	0	0	21
Night monkeys (*Aotus zonalis*)	1	28	5	1	35
Total	12	181	10	6	209

mates has been considered separately from that concerning nonprimates. Animal species were identified in some instances by the Smithsonian Institution, Washington, D. C. and in others in accordance with the descriptions of Goldman (1920).

RESULTS OF TESTS ON PRIMATE BLOODS

In the protection tests employing primate bloods, a total of 12 positives was encountered among the 209 monkeys examined. Five of 85 squirrel marmosets, 4 of 24 red spider monkeys, 2 of 20 black spider monkeys and one of 35 night monkeys gave positive tests. Spider and howler monkeys proved most satisfactory for testing both because their large size facilitated bleeding and because their sera yielded clear-cut positive or negative results that could be reproduced with utmost fidelity both with serum stored for varying periods of time and with serum freshly obtained by rebleeding at appropriate intervals. Squirrel marmosets, night monkeys and whiteface monkeys gave a number of inconclusive and unsatisfac-

tory results. These animals also showed the greatest number of spontaneous parasitic and bacterial infections. Inconclusive results on the first bleeding were

TABLE 2

Places of origin of primates tested and year of capture

PLACE OF ORIGIN	NO. RECEIVED PER YEAR			
	1949	1950	1951	Total
Panama, west of Canal				
Province of Chiriqui				
Squirrel marmosets	0	1	0	1
Whiteface monkeys	0	1	5	6
Howler monkeys	0	2	2	4
Province of Bocas del Toro				
Red spider monkey	1	0	0	1
Provinces of Veraguas, Cocle, Herrera and Los Santos				
Red spider monkeys	2	0	1	3
Whiteface monkeys	1	0	2	3
Howler monkeys	0	0	1	1
Province of Panama, west of Canal				
Squirrel marmosets	7	5	4	16
Red spider monkeys	1	0	2	3
Night monkeys	0	1	1	2
Canal Zone				
Squirrel marmosets	0	0	2	2
Howler monkeys	1	0	1	2
Panama, east of Canal				
Province of Colon, east of Canal				
Squirrel marmosets	0	1	0	1
Red spider monkeys	1	1 (1)*	2	4 (1)
Whiteface monkeys	0	0	1	1
Province of Panama, east of Canal				
Squirrel marmosets	21 (2)	10	33 (3)	64 (5)
Red spider monkeys	5 (3)	3	5	13 (3)
Black spider monkeys	1	6 (1)	6	13 (1)
Whiteface monkeys	0	2	5	7
Howler monkeys	3	4	5	12
Night monkeys	8	6	19 (1)	33 (1)
Province of Darien				
Black spider monkeys	2 (1)	2	2	6 (1)
Whiteface monkeys	1	1	1	3
Origin Unknown				
Squirrel marmosets	0	1	0	1
Black spider monkeys	0	1	1	2
Whiteface monkeys	2	0	3	5
Sum total	57 (6)	48 (2)	104 (4)	209 (12)

* Numbers in parenthesis represent numbers of positive reactors obtained.

obtained in a total of 18 monkeys, including 7 squirrel marmosets, 4 whiteface monkeys and 7 night monkeys. Unsatisfactory results were obtained on first bleeding in 10 of the animals, including 4 squirrel marmosets, 5 whiteface mon-

keys and one night monkey. We were able to repeat the test after rebleeding in 19 of these 28 animals giving an initial inconclusive or unsatisfactory result. Of 13 originally classified as inconclusive, 8 became negative, 4 continued inconclusive and one gave an unsatisfactory result. Of 6 classified originally as unsatisfactory, 4 became negative, one gave an inconclusive test and one was again unsatisfactory. These results are tabulated in Table 1.

In Table 2 are presented data concerning the places where the various monkeys were trapped and the year of their acquisition by the laboratory. A total of 40 monkeys was received from the section extending west of the Canal Zone toward the Costa Rican border. Thirty-nine of these were from the Pacific side of the Continental Divide and only one from the Atlantic side. There were no positives in this group nor among 4 monkeys obtained from the western part of the Canal Zone. A total of 157 monkeys was received from the section extending east of the

TABLE 3

Age of monkeys tested

SPECIES OF MONKEY	ADULTS		JUVENILES	
	No. tested	No. positive	No. tested	No. positive
Squirrel marmosets	72	5	13	0
Howler monkeys	2	0	17	0
Red spider monkeys	8	0	16	4
Black spider monkeys	4	1	17	1
Whiteface monkeys	12	0	13	0
Night monkeys	29	1	6	0
Total	127	7	82	5

Canal Zone toward the Colombian border. Of these, 12 gave a positive test. The exact place of origin of 8 was unknown. The great majority of all monkeys (142) were received from the Province of Panama, east of the Canal. Fifty-seven primates were acquired in 1949, of which 6 gave a positive test: 48 in 1950 with 2 positives and 104 in 1951 with 4 positives.

In Table 3 is presented a classification of monkeys in accordance with their ages. The large majority of marmosets and night monkeys were adults when received. Of the larger monkey species the majority were juveniles. This preponderance of juveniles among the spider, howler and whiteface monkeys resulted of course in a lower percentage of positives than would be found in a balanced group. However, positive results among them have greater epidemiological significance as they indicate that yellow fever had been active recently in the areas in question. Of 6 positive spider monkeys 5 were classified as juveniles, probably 8 to 18 months of age when purchased. Three of these were received in 1949 and 2 in 1950. Four were from the vicinity of the Bayano River and one from the vicinity of the Gatuncillo River. As the studies of Soper and coworkers (1938) indicate that antibodies acquired passively from the mother are lost in 6 months, it may be concluded that yellow fever was active in these areas in 1948 and 1949.

RESULTS OF TESTS ON NONPRIMATE BLOODS

A tabulation of the results obtained in protection tests with nonprimate bloods is presented in Table 4. A total of 58 animals of 15 different species was tested, 23 having been received in 1949, 15 in 1950 and 20 in 1951. A strongly positive result was obtained with the blood of one pygmy anteater, *Cyclopes didactylus*, an adult male captured in Curundu in the Canal Zone in April 1951. Three others gave a negative and one an unsatisfactory test. Two agoutis, *Dasyprocta punctata*, a male and female juvenile respectively, both received from the vicinity of the Sabalo River in Darien in June 1951, gave a positive result when first bled on June 9. They were both rebled on September 18 when an inconclusive result was

TABLE 4

Results of intraperitoneal protection test in Swiss mice with nonprimate bloods

SPECIES OF ANIMAL	POSITIVE	NEGATIVE	INCONCLUSIVE	UNSATISFACTORY	TOTAL
Three-toed sloth (*Bradypus griseus griseus*)	0	10	1	0	11
Coati (*Nasua narica panamensis*)	0	13	0	0	13
Agouti (*Dasyprocta punctata*)	2*	9	0	0	11
Pygmy anteater (*Cyclopes didactylus dorsalis*)	1	3	0	1	5
Great anteater (*Myrmecophaga tridactyla centralis*)	0	1	0	0	1
Opossum (*Didelphis marsupialis etensis*)	0	6	0	0	6
Woolly opossum (*Caluromys laniger*)	0	1	0	0	1
Water opossum (*Chironectes panamensis*)	0	1	0	0	1
Raccoon (*Procyon cancrivorus panamensis*)	0	2	0	0	2
Kinkajou (*Potos flavus isthmicus*)	0	1	1	0	2
Paca (*Cuniculus paca virgatus*)	0	1	0	0	1
Collared peccary (*Pecari angulatus bangsi*)	0	1	0	0	1
Tapir (*Tapirella bairdii*)	0	1	0	0	1
Grison (*Grison canaster*)	0	0	0	1	1
Porcupine (*Coendou rothschildi*)	0	1	0	0	1
Total	3	51	2	2	58

* First test.

obtained. One was bled a third time on February 7, 1952 when a totally negative result was given. The first test was preformed after the sera had been stored in the frozen state for 2 weeks, the second and third tests within 24 hours of bleeding. Considerable experimental work has been done with various species of agoutis. Bugher and coworkers (1944) found this animal to be susceptible to yellow fever, as also *Cyclopes didactylus*. Laemmert (1948) found evidence of formation of antibodies in the agouti after inoculation with virus but could not demonstrate circulation of the virus.

DISCUSSION

In the present survey of 209 monkeys captured in various sections of Panama, a total of 12 positives or 5.7 per cent was obtained. The specificity of the reaction

in suitably drawn, noncontaminated primate bloods is generally acknowledged. However, the proportion of positive monkeys in this series must be considered low. Haddow *et al.* (1947) found 61 per cent of positive bloods among 150 primates studied in Uganda. Kumm and Laemmert (1950) found an average of 12.4 per cent positives among a total of 5857 monkeys examined in Brazil, and Bugher (cited by Taylor 1951) in 67 per cent of 183 primates captured or shot in a postepidemic period and 70 per cent of 44 primates in an epidemic period in the Restrepo-Villavicencio region of Colombia. However, the number of human cases in these countries has been high in comparison to Panama where the disease had remained clinically unrecognized for more than 40 years prior to the recent outbreak. Clark (1950) found 51.7 per cent positive among 201 monkeys shot in various localities east of the Canal and 30.3 per cent positive among 224 monkeys shot in localities west of the Canal. The great preponderance of juvenile animals among the larger monkeys in our series may account in part for the discrepancy. Also the use of the less sensitive intraperitoneal test and the very irregular geographic distribution of the specimens received may have influenced our results. In general, the present findings, though limited in scope as noted above, correspond to what we know of the epidemiology of the disease here. No positives were found among 44 primates from the Canal Zone and the Provinces of Chiriqui, Herrera, Cocle, Veraguas and Panama west of the Canal, in which areas human cases of yellow fever have not been recorded, and in which Kumm and Crawford (1943) found only negative human sera in a survey conducted in 1943. Ten immune monkeys were obtained from the Province of Panama, east of the Canal, where jungle yellow fever has been shown to be endemic in recent years and where the first cases of the present outbreak occurred in 1948. One immune monkey was received from the Province of Darien, and one from the Province of Colon east of the Canal where 2 cases of yellow fever occurred in 1949. Unfortunately, no specimens were received from the Province of Colon west of the Canal and only one from Bocas del Toro where human cases were registered in 1951, from one of which the author isolated a strain of virus of high antigenicity but low pathogenicity in monkeys.

Since the specificity of protection tests with nonprimate bloods has been shown to be affected by a number of variables by Koprowski (1946), no attempt will be made to interpret the temporarily positive results obtained in 2 agoutis and the positive result in one pygmy anteater until further experimental work has been completed.

CONCLUSIONS

Among 209 primates from various sections of Panama, a total of 12 gave a positive reaction in the intraperitoneal protection test for yellow fever in Swiss mice.

Of 58 other animals, one pygmy anteater and two agoutis also gave a positive test, the reaction in the latter species being temporary.

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COMMENTS ON THE TREATMENT OF TRACHOMA WITH AUREOMYCIN

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The idea of using aureomycin in the treatment of trachoma came from a report which Dugger presented in 1948 before the Academy of Sciences in New York. Indeed, the antibiotic properties of aureomycin are of such wide range that it offers many possibilities for trial against both viral and rickettsial diseases. *In vivo*, it acts not only against the gram-positive organisms, like penicillin, but also against the gram-negatives like streptomycin and chloromycetin, with the exception of the proteus group. Apparently, it is effective against organisms which are resistant to both penicillin and streptomycin and in addition it is reported to be effective against the rickettsial diseases and the viruses of psittacosis and lymphogranuloma venereum.

In the therapeutic application of our laboratory studies, it naturally came to mind to use aureomycin in the treatment of trachoma. As a result of the extensive research of Nataf (1950), Burnet, Bland, Guened and Biette, it is now known that the causal agent of trachoma presents indisputable analogies both with the rickettesial group and the viruses. Braley and Sanders (1948), Montinho, Grillo and Moura (1949), Duke-Elder *et al.* (1950) and Nataf have all reported more or less successful results from the use of aureomycin in the treatment of trachoma during the years 1948–1949. Also, at a meeting of the Society of Ophthalmology of Paris in February, 1950, Desvignes reported good results from the treatment of trachoma with this drug.

At the Ophthalmologic Institute in Hanoi we have used aureomycin ointment (aureomycin—HCL 1.0 gm. per gm.) in the treatment of 22 cases of trachoma. Considering the limited amount of ointment at our disposal and the necessity of confining our experiment within reasonable limits, it was not possible to include all forms of trachoma in the series.

The cases of trachoma observed at the Ophthalmologic Clinic are rarely in the early stages. Its indolence and subtle development in infants cause the initial stages to go unheeded by the patient. At a more advanced stage, a period when granulations become apparent on a level with the conjunctiva, the patient goes voluntarily to a doctor for examination. The most advanced stage is characterized by the transformation of the tarsal conjunctiva into a fibrous cicatricial tissue. This is the most resistant form of trachoma and all attempted treatment with antibiotics fails.

As a result of our investigations, we conclude that there are two important stages of trachoma which respond readily to treatment with antibiotics: (1) the initial stage, which injures only the adenoid layer, and (2) the evolutive stage, where granulations begin to advance to the conjunctival level. We are primarily interested in the effect aureomycin has on these two stages. Previous studies show that the simple application of aureomycin ointment does not

always give the desired results. In order to be effective, aureomycin, like any other antibiotic or antiseptic, must penetrate deeply into the tissue.

TECHNIQUE

First, it is necessary to abrade the granulations. After having anesthetized the conjunctiva by administering several drops of 4% cocaine, the upper eyelid is turned up and the patient is asked to look downward. An assistant everts the lower eyelid in the same way, putting ectropion in the tarsal conjunctiva and the inferior cul-de-sac. The two eyelids so turned touch each other and the granulations which appear are carefully scraped with a small, sharp curette. This is followed by a generous application of aureomycin ointment which is repeated in the afternoon. The curettage is performed every three days followed by the application of the ointment which, because of the exfoliation of the conjunctival epithelium, directly affects the underlying adenoid tissue.

RESULTS

In spite of the discomfort caused by the curettage, the symptoms subside very rapidly in most cases and the patient feels much improved at the third treatment. A sensation, like that of a grain of sand, frequently disturbs the patient upon awakening, while heaviness of the eyelids and photophobia persist for a week to ten days. The very heavy secretions, which appear the day after each scraping, tend to disappear completely. Noticeable also is the remarkable regression of phenomena such as lacrimation and enlargement of conjunctival vessels. By continuing this treatment for two weeks to 20 days at the maximum, one can obtain in almost every case a simple, fine cicatrization. Granulations disappear and only a diffuse redness remains which disappears completely after a week.

It is very rare for the tissues of the eye to return to a completely healthy condition after an attack of trachoma. There is, however, a period of remission during the time the trachomatous process is being cicatrized. At this time, we consider the patient healed or at least immune to new infection. The patient no longer shows visible granulations, or additional signs of inflammation. Conjunctiva, chorion, and tarsus are no longer infiltrated. Granulations are flattened and replaced by very soft cicatricial meshes, which are sometimes not seen even upon direct examination.

Outlined below are the results of treatment on 22 patients, aged 3–12: 2 cases of cicatricial trachoma—slight improvement of subjective symptoms but no modifications of anatomic lesions; 11 cases of evolutive trachoma—great improvement of symptoms and signs with very soft cicatrization on the conjunctiva; 9 cases of initial trachoma—complete disappearance of subjective symptoms with fine, almost invisible, cicatrization of the conjunctiva.

MODES OF ACTION

We might ask ourselves what is the mode of action of aureomycin ointment after curettage. Is it primarily a mechanical action resulting from the removal of

the granulations, or is it strictly the antibiotic action of the ointment which itself is specific against the trachoma virus?

We believe that the mechanical effect must be significant because of the many surgical methods which have been utilized with benefit in the past such as brushing, curettage, scarification, crushing and expression of granulations and blepharoxysis. In this method of treatment, the mechanical action is simply reinforced by the strongly antibiotic effect of the aureomycin which acts directly through the lesions produced by the scraping. We have tried applying ointment alone to two patients having initial trachoma. We were not surprised when after two weeks of treatment, the anatomic lesions were unchanged. After curettage and the application of ointment, the good results obtained in other patients were confirmed in these two controls.

It is known that with other products such as penicillin, sulfonamides or chaulmoogra oil, favorable results are obtained using the same technique. But aureomycin acts more rapidly, sometimes causing almost complete disappearance of lesions in less than a week in the case of early initial trachoma.

These experiments involving a limited number of cases do not enable us to formulate a definite conclusion; however, the good results from the use of aureomycin on trachomatous granulations, after curettage in the evolutive and initial stages, are remarkable because the antibiotic ointment does not have any caustic or irritating action.

CONCLUSION

The application of aureomycin ointment to trachomatous lesions after abrasion of granulations seems to be a method of treatment of trachoma in the initial and evolutive stage which might well be recommended in preference to other techniques.

Aureomycin is well tolerated by young patients. It does not bring about complete restoration to a healthy condition but rather accelerates cicatrization of trachomatous lesions.

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TERRAMYCIN IN THE TREATMENT OF TRACHOMA

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The wide range of activity of terramycin, whose action is so remarkable on the principal gram positive and gram negative bacteria, the rickettsias and certain viruses, has led various physicians to test this drug in the treatment of trachoma. We are grateful to Dr. Winkelstein, STEM, Public Health Department, for providing a sufficient amount of this new antibiotic for experimentation, and we have tested its effects on a group of patients infected with florid trachoma in the more active stages of the disease and trachoma with complications. Our methods were similar to those used in previous tests with chaulmoogra oil, penicillin and aureomycin, which consist of applying the medicine to the granulations after abrasion by a curettage deep enough to cause bleeding.

MATERIALS

We are indebted to the Pfizer Laboratories of New York for making available an ointment of 1:1,000 crystalline-terramycin chlorohydrate in suspension in a petrolatum base, and an aqueous collyrium of crystalline-terramycin chlorohydrate with sodium borate and sodium chlorite made up at the time of use.

PROCEDURE

We have used terramycin on 14 subjects from 8 to 25 years of age whose trachomatous lesions can be classified in 4 categories:

1. Evolutive trachoma with florid, luxuriant and sometimes hypertrophic granulations (7 cases);
2. Sclero-cicatricial trachoma without corneal complications (2 cases);
3. Cicatricial trachoma with pannus tenuis (2 cases) and pannus crassus (1 case);
4. Cicatricial trachoma with intensive secondary infections (2 cases).

The trachomatous lesions submitted to the action of the antibiotic were given a preliminary curetting to break up the granulations and allow impregnation with the drug. After each curetting the lesions were packed with wet sterile cotton. An abundant instillation of terramycin collyrium was then made, followed within a minute by an application of terramycin ointment.

After the exfoliation of the conjunctival epithelium and the rupture of the granulations, the antibiotic is given an opportunity to act directly on the subjacent adenoidal tissue, a pre-eminently vascular zone with a network of capillary vessels formed largely by the junction of the terminal branches of the palpebrals constituting the origin of the pannus's new-formed vessels.

Curettage was performed every 3 days. Instillations of collyrium and applications of ointment were made 4 times a day. Within the whole period of treatment, the patients received no other medication except that given locally. In

the two cases which presented secondary infections, the curettage was made only when inflammatory phenomena had completely regressed.

CLINICAL RESULTS

The most striking effect of the treatment with terramycin is the rapid regression of the symptoms of trachoma, involving an obvious decrease of photophobia and watering (Plates 1 and 2). In our two cases of trachoma intensely infected with staphylococci, streptococci and Koch-Weeks bacilli, the disappearance of acute inflammatory phenomena was obtained in less than 4 days. For this effect alone, the new antibiotic would deserve its place in the antitrachoma arsenal with aureomycin. The consequences of secondary infections are well known for they predispose to ulcerations and to vascular infiltrations of the cornea.

But does terramycin cure trachoma? It must be recognized that it is difficult to state the criteria of recovery in a disease with chronic evolution. However, it is generally thought that in the presence of stabilized cicatrization, with the flattening of granulations and no superadded inflammatory signs, trachoma can be considered as cured from the clinical and social standpoints.

All the patients subjected to the experiment underwent a treatment of from 24 days to 2 months. Analysing successively the different elements observed in connection with the nature of the trachomatous process, we found that:

1. In evolutive trachoma the florid granulations generally subsided by the 30th day under curettage and terramycin. After 2 weeks of treatment, hyperemia decreased. There was also a gradual decrease in discharges. Dense and hypertrophic granulations flattened slowly but progressively. The mechanical effect of curettage is important in therapy.

By destroying all superadded infection and by its possible antivirus action, the antibiotic helps the cicatricial progress. The reduction of granulations in certain cases can lead to the formation of fine cicatrices presenting the aspect of a somewhat velvety conjunctiva.

It is difficult to determine whether cases clinically recovered are immune from new infections since in tropical countries trachoma is a familial disease. Once returned to his environment, the patient runs the chance of being reinfected again due to sources within the household and to general lack of hygiene.

2. With the subjects presenting cicatricial trachoma, the action of terramycin on sclerous lesions can be considered negligible, even when the lesions are submitted to a long and elaborate curettage. However, an improvement of subjective signs can be observed in these patients due to the effect of terramycin on latent infections common in trachoma patients. The improvement consists in the disappearance of the serous secretions that glue the eyelids in the morning, and a decrease of watering and photophobia.

3. As far as corneal alterations are concerned, the results obtained are very encouraging. However, the improvement is slow and depends upon the length of treatment. In pannus tenuis, the resorption of the vessels can be observed towards the end of the 2nd week when the cicatricial evolution begins to progress at the level of the upper conjunctival fold, a predisposed zone for pannus. Pannus

TABLE 1

Protocol of observations

NO.	PATIENT, AGE, SEX	LESIONS	METHOD OF TREATMENNT	DURATION OF TREATMENT	RESULTS
1	P.T.D. 19, F.	Evolutive trachoma with exuberant granulations and pannus tenuis (Plate 1)	Curettage every 2 days; drops and ointment 4 times a day	2 months	Pannus completely disappeared, good cicatrization of granular lesions
2	N.T.B. 23, F.	Evolutive trachoma, hypertrophic at the conjunctival fold	Curettage, drops and ointment	1 month & 20 days	Cicatrization of the lesions
3	D.T.H. 10, F.	Evolutive trachoma with exuberant granulations; blepharitis-pannus crassus	Curettage, drops and ointment	1 month & 4 days	Good cicatrization. Pannus cleared up
4	N.T.H. 16, F.	Evolutive trachoma; big granulations	Curettage, drops and ointment	2 months	Good cicatrization of lesions
5	N.T.D. 15, F.	Hypertrophic granulations at upper lids. Pannus tenuis	Curettage, drops and ointment	1 month & 6 days	Pannus disappeared, cicatrization of lesions
6	D.N.T. 15, M.	Big granulations, exuberant and very tough (Plate 2)	Curettage, drops and ointment	2 months	Slight reduction of volume of granulations
7	D.T.B. 14, F.	Big granulations, very tough and very dense	Curettage, drops and ointment	1 month & 26 days	Slight decrease of volume of granulations
8	N.V.K. 13, M.	Cicatricial trachoma	Curettage, drops and ointment	24 days	Improvement of subjective signs. Trachoma unchanged
9	N.T.M. 17, F.	Cicatricial trachoma. Pannus tenuis-blepharitis	Curettage, drops and ointment 4 times a day	1 month & 10 days	Blepharitis cured in 5 days. Pannus cleared up. Trachoma unchanged
10	N.T.T. 11, F.	Hypertrophic cicatricial trachoma. Trichiasis upper lid	Curettage, drops and ointment. Entropion operation	1 month & 20 days	Improvement of functional signs. Trachoma unchanged
11	N.V.A. 8, M.	Cicatricial trachoma	Curettage, drops and ointment	1 month	Improvement of functional signs. Trachoma unchanged
12	N.T.D. 25, F.	Exuberant cicatricial trachoma. Pannus crassus	Curettage, drops and ointment	1 month & 28 days	Slight clearing up of pannus. Trachoma unchanged
13	P.H.T. 11, M.	Evolutive trachoma with obvious secondary infections. Very dense granulations (Weeks bacilli, streptococci)	Drops and ointment, then curettage from the 8th day	28 days	Inflammatory signs disappeared after about a week. Good cicatrization
14	N.T.H. 18, F.	Infected cicatricial trachoma, mucopurulent secretions, slight chemosis, swollen lids (streptococci)	Drops and ointment then curettage from 5th day	24 days	Inflammatory sign disappeared toward 3rd day. Cicatrix unchanged

crassus begins to display a partial clearing up towards the 5th week. Instead of this long term treatment, we prefer the surgical processes (peritomy, heat-cauterizations) which gave us the best results. Within the limits of experimentation, the action of terramycin on pannus was less efficient than that of aureomycin under similar conditions.

TOLERANCE

Generally, no intolerance to terramycin was observed. Like aureomycin, terramycin in local application caused no inflammatory or painful reactions. Intolerance of an allergic character did not occur. We did not notice those palpebral eczemas such as were observed in certain patients during the local treatment with penicillin. It is also worth noting that in some of our patients, who presented lesions of confirmed blepharitis, regression of inflammatory phenomena was obtained in less than 10 days after applications of terramycin ointment.

DISCUSSION

The tests carried out are still too limited in number and duration to allow precise evaluation. Nevertheless, the first impression that arises from this therapeutic study is that terramycin has a remarkable action on the secondary infections that complicate trachomatous lesions. In that respect, it seemed to us that the results obtained by terramycin and aureomycin are equal. Trachoma is a chronic disease with a consistent evolution under normal conditions towards spontaneous cicatrization. Its intensity and aggravation depend largely upon superadded infections. When the latter can be stopped in a rapid and sure way, the disease is considerably impeded in its development.

That terramycin can act on trachoma virus has not yet been determined definitely. Clinically, when a test drug with no astringent action is able to eliminate the sero-mucous secretion peculiar to trachomatous lesions, it can be assumed that its effect is positive on the agent of trachoma. Both terramycin and aureomycin produce such a result when used in trachoma therapy. However, it cannot be assumed that terramycin is a specific drug for trachoma, because of elements the antibiotic is unable to reduce. The evidence of it are those florid, dense and hypertrophic granulations that the elaborate and continued application of terramycin, subsequent to the curettage of lesions, cannot cure. It is doubtful, therefore, that terramycin kills the trachoma virus.

CONCLUSIONS

Terramycin was used in severe trachoma cases to evaluate its effect. The results indicated that terramycin used locally in trachoma, after elaborate curettage of the lesions, gave encouraging results comparable to those obtained with aureomycin.

Against secondary infections, terramycin has a rapid and dramatic action (3 to 5 days), far better than that of penicillin and the sulfonamides.

In evolutive trachoma, and specially in florid forms, terramycin assists in

causing suitable cicatrization, but its action is slow (6 weeks to 2 months) and seems to be largely aided by the mechanical effects of the abrasion of the granulations by curettage. Complete restoration has not been obtained.

Against minor complications of trachoma (blepharitis, pannus tenuis) terramycin exerts a favorable action.

Terramycin does not cause the resorption of sclerous lesions It improves the subjective symptoms by reducing secretory manifestations.

In local application, terramycin is very well tolerated by the patients. In spite of repeated use, it is not irritating to the bulbar conjunctiva or the corneal epithelium. Allergic reactions were not observed.

Terramycin cannot be regarded as a specific drug for trachoma, but it unquestionably constitutes a valuable contribution, with a well-defined effectiveness in trachoma treatment. Its use is to be considered in all cases of trachoma on account of its quick effects on the conjunctival secretions, which are a source of secondary infection.

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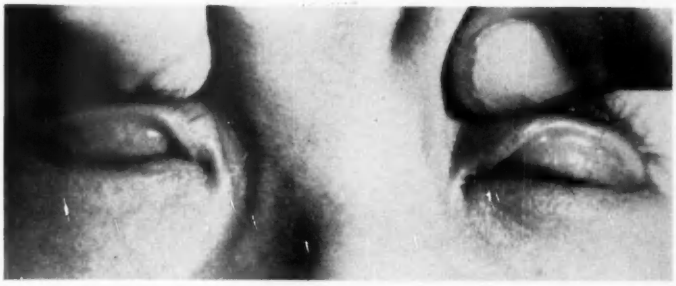
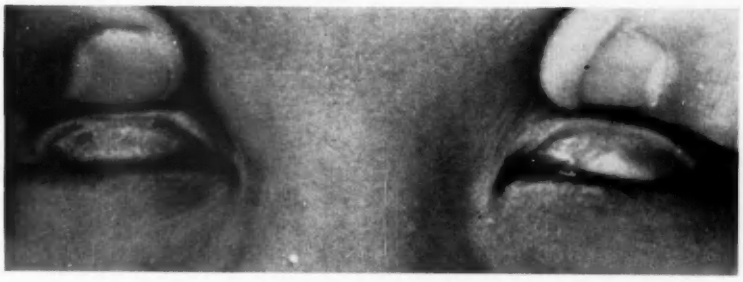
PLATE 1. Case 1. P. T. D., 19 years, female. Before treatment, June 12, 1951 (florid, evolutive trachoma). One month later, July 12, 1951. End of treatment, August 30, 1951.





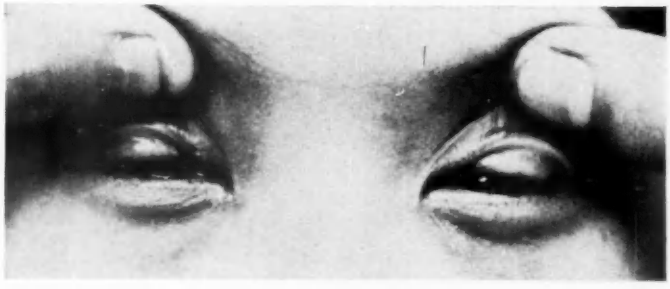
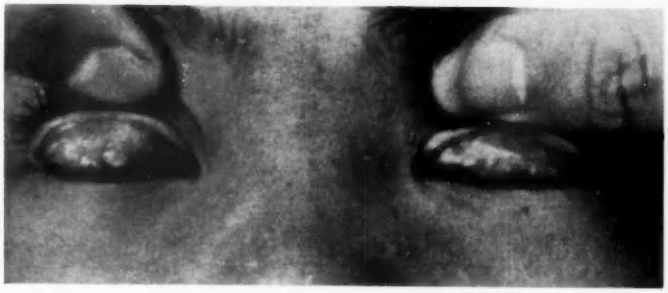
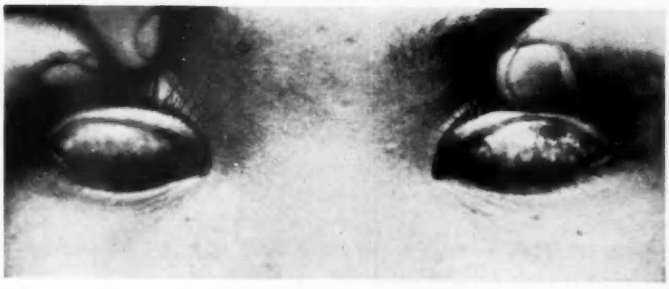


PLATE 2. Case 6. D. N. T., 15 years, male. Before treatment June 20, 1951 (evolutive, exuberant trachoma). 27 days later, July 12, 1951. End of treatment, August 17, 1951.





THE BEHAVIOR OF *RICKETTSIA AKARI* IN THE BODY LOUSE AFTER ARTIFICIAL INFECTION

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Rickettsialpox, belonging to the group of rickettsial diseases and characterized by skin eruptions, was first discovered in 1946 in New York and has since been reported in Boston by Pike *et al.* (1950). This disease presents a number of interesting biological problems. In an astonishingly short time the etiology and epidemiology of the disease were elucidated. The agent, *Rickettsia akari*, was cultivated in the chick embryo and its immunologic relationships established, mainly by Huebner and his associates (1946) as reviewed by the German author Kirsch (1949). The reservoir of the disease was found to be the house mouse *Mus musculus*, and the transmitter a mite of the family of *Laelaptidae*, *Allodermanyssus sanguineus* Hirst. It is not yet known whether there are other vectors. Philip and Hughes (1948) demonstrated experimentally, that the tropical rat mite *Liponyssus bacoti* Hirst can transmit *R. akari*. In one test rickettsiae were found in the nymphs of the F_1 generation, possibly by infection of the ovaries.

In the course of investigations of the behavior of various rickettsiae in arthropods we have examined the relationship of *R. akari* to the body louse. It was of special biological interest to discover whether the body louse is susceptible to these rickettsiae at all and how it reacts to the infection as compared with other rickettsiae.

MATERIAL AND METHODS

Dr. Italo Archetti from the Istituto di Sanitá Pubblica in Rome was kind enough to send me the Kaplan strain of *R. akari* from the Rocky Mountain Laboratory, Hamilton, Montana, in the form of yolk sac tissues dried in September 1949. With this material we first inoculated a series of mice intraperitoneally. As soon as there were sufficient rickettsiae in the smears of the mouse organs, we inoculated body lice (*Pediculus humanus humanus* L.) rectally by the method of Weigl and intracoelomically with infectious material from the mouse, using small glass capillaries. The intracoelomic infection was carried out only in females through the vagina. For each louse we used approximately 0.3 mg. of the infectious suspensions. The lice which withstood the infection well were kept in metal boxes and fed on the author's leg to which the boxes were strapped one hour every day. Between the blood-meals the lice were kept in an incubator at 31°C.

To follow the results, smears of the stomachs of lice rectally inoculated were made at regular intervals, and stained by the method of Giemsa. The lice intracoelomically infected were examined by means of smears of the coelomic liquid. Little droplets of the coelomic liquid can easily be obtained without

damage to the louse by amputation of a leg. After it was discovered that rickettsiae do multiply in the louse, special attention was paid to their localization in specific organs and in the cells themselves. For this purpose careful histological investigation became necessary. The stomachs were either taken out in a drop of broth and fixed, or the whole abdomen was put into the fixing solution after removing the head and extremities. We used Carnoy's fixing solution (6 parts absolute alcohol, 3 parts chloroform, 1 part glacial acetic acid). The material was treated with methylbenzoate, embedded in paraffin and then cut into longitudinal and cross sections of 3–4 μ which were stained by the moist Giemsa method and differentiated with acetone.[1]

As soon as multiplication of *R. akari* in the louse was established, the rickettsiae were again transmitted to healthy lice by injecting them rectally with suspensions of the stomachs of infected lice to make sure that the rickettsiae could be maintained in the louse by serial transfer. Altogether 6 louse passages were carried out by rectal inoculation without any essential change in the general picture, 35–40 lice being inoculated in each lot. Since the behavior of the rickettsiae after intracoelomic inoculation was rapidly revealed, only 2 series of 35 females each were infected by this method. Finally, the virulence and pathogenicity to mice of the rickettsiae from the lice were tested by intra-abdominal inoculation of stomach suspensions, coelomic liquid, and feces of the lice.

The lice would not feed on the mouse, so we were not able to infect them in the natural way by letting them feed on sick mice. We therefore inoculated 2 series of lice rectally with the blood of acutely ill mice, diluted with broth. Whether the infectious material reached the stomach via the rectum or the esophagus is perhaps immaterial, the important point being to discover whether the diluted inoculum contained enough rickettsiae to establish infection in the louse. These lice, like the others, were then nourished by feeding them on man.

BEHAVIOR OF THE STRAIN IN MICE

Mice, inoculated with the Kaplan strain of *R. akari* preserved in the form of dried yolk sac tissues, showed no clinical symptoms on the first passage, although spleen and liver preparations revealed isolated, pale rickettsiae. The second passage, initiated with spleen and liver suspensions, gave rise to clinical symptoms on the 7th day and one mouse died on the 8th day. The pathological picture was typical and did not change materially in subsequent transfers. Clinical symptoms became progressively more severe and at the fifth passage all the mice were dead by the 6th day. Smears from the organs, especially the liver, contained large numbers of rickettsiae as early as the 2nd passage. Intraperitoneal inoculations of spleen and liver suspensions were kept up for 6 passages, but beginning with the 4th passage, mice could also be infected intranasally by inhalation of spleen and liver material. These mice developed symptoms after 3 days and died as a rule on the 4th with typical pneumonitis. The lungs showed notable pathologic changes and smears contained large numbers of

[1] I am indebted to Dr. W. Behrenz for making the sections and to Miss Magdalene Kerner for her valuable technical assistance.

rickettsiae. While the strain could have been maintained in the lungs, this procedure was substituted, after 2 passages, by the inoculation of 6 to 7 day old chick embryos with organ suspensions from the 4th and 6th passages, by Cox's method. The embryos died in from 3 to 6 days, and smears from the yolk sacs contained rickettsiae in ample numbers. Thus the strain behaved normally, was quite virulent, and had evidently regained its original properties.

THE BEHAVIOR OF *R. AKARI* IN THE LOUSE

The external phenomena. Since the peritoneal smear of a mouse of the second passage contained a sufficient quantity of rickettsiae, the first series of lice was rectally inoculated with the peritoneal exudate diluted in broth. At first nothing unusual was found in the infected lice, but by the 7th day rickettsiae could be located beyond any doubt in smears of the stomachs. In later passages

FIG. 1. Smear of the stomach of a louse, rectally infected with *R. akari*. 8th day after inoculation. Epithelial cells with rickettsiae. Staining in all figures is with Giemsa. All figures except Fig. 7 are magnified 1200 times.

they could be found after 6 days but not earlier. Their number increased continuously in the following days and reached a maximum after 10 to 12 days.

The rickettsiae were rather evenly distributed in the smears (Fig. 1). Their color was darker than in the smears of the mouse. Coccoid forms and in particular plump, double rod-shaped forms predominated. Slender forms, thin and bright in the central portion, or longer chains such as are the rule after an infection with *R. prowazekii* or *R. mooseri* during the first few days, were never observed. The rickettsiae did not lie in compact and dense heaps, but were scattered and isolated. They strongly resembled *R. quintana* in size, shape, color and distribution. The staining of the poles was not noteworthy, especially at first.

After 7 days, rickettsiae appeared in the feces of the lice, but the number was relatively small throughout the test, constantly fluctuating and never reaching the quantities characteristic of *R. prowazekii* or *R. quintana*. The reasons will be discussed below.

In the first two lots of lice, none were obviously damaged by the rickettsiae.

In all, only 11 of 70 infected lice died after 8 to 15 days in one series, and 7 after 7 to 16 days in another. The death of these lice was probably caused by the rickettsial infection. The surviving lice of the first test were dissected after 17 days and at that time still contained many rickettsiae. On the 13th day 4 stomachs were emulsified in broth and rectally injected into a new lot of lice. In this passage the same phenomena reappeared as those described above: the lice had demonstrable infections after 7 days and contained large numbers of rickettsiae in the stomach. In this manner the strain was kept without incident for 6 passages over a period of 12 weeks. The experiments were interrupted after the sixth passage because the behavior of the rickettsiae and the reaction of the lice did not alter materially. In this way *R. akari* can doubtless be maintained in the louse indefinitely.

It was remarkable not only that *R. akari* was able to multiply rapidly and consistently in the lice, practically all of which became infected, but also that they caused no perceptible injury to most of the lice. Some infected lice remained

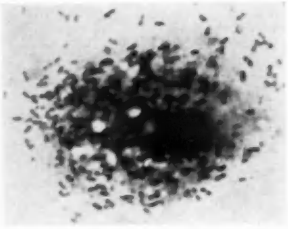

FIG. 2. Smear of the coelom liquid of a louse, intracoelomically infected with *R. akari*. 10th day after inoculation.

alive 3 or 4 weeks, i.e. their normal lifetime was not essentially shortened by the rickettsial infection.

Lice were easily infected with blood from a sick mouse and large numbers of rickettsiae were found after 9 days, although the lice were kept at room temperature for 3 days. Practically all the lice became infected and behaved in the same way as after inoculation with organ suspensions, showing little damage from the infection.

Intracoelomic injections, however, had different consequences. In earlier experiments the coelom of the body louse had proved to favor the growth of various rickettsiae (Weyer 1950). This is also true of *R. akari* which multiply rapidly and flourish under these unusual conditions (Fig. 2 and 3.) In the first experiment the lice were injected with the peritoneal exudate of a mouse; in the second, with the ground stomachs of some positive lice. After 5 to 6 days there was clear evidence of rickettsiae in the coelomic liquid. Their number did not increase remarkably during the first 8 to 10 days, but then rapid multiplication began and the whole coelom was flooded with the organisms, damaging the lice and killing them in a short time. The abdomen was swollen and frequently the lice had a reddish color. The last lice of the first lot were sacrificed after 11

days and a series of mice were successfully infected intraperitoneally with the coelomic liquid taken from four lice. In the second lot no lethal effect was observed until the 11th day, after which the coelomic liquid of both dead and living lice was densely filled with rickettsiae. The surviving lice were dissected after 14 days.

Internal phenomena and topography. The stomach smears did not indicate with absolute certainty whether the rickettsiae in the louse were intra- or extracellular (Fig. 1). While the regularity of arrangement seemed to indicate an intracellular position, the scattered distribution of the relatively dark stained rickettsiae frequently resembled the extracellular development of *R. quintana*. Also the early appearance of rickettsiae in the feces seemed to prove this. In later stages, however, a notable vacuolisation of the stomach cells was noticed

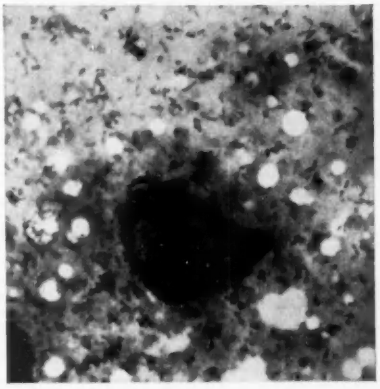

FIG. 3. Smear of the coelom liquid of a louse, intracoelomically infected with *R. akari*. 10th day after inoculation. Accumulation of rickettsiae in blood cells.

in the smears and there seemed to be rickettsiae in the vacuoles. However, their position could not be definitely determined until the lice had been examined histologically at different stages of the infection (Fig. 4 to 7).

These examinations showed that, with rectally inoculated lice, the rickettsiae multiply only in the cells of the midgut and do not invade the rest of the alimentary canal or other tissues. Unlike *R. quintana* which is found on cell surfaces and in the lumen of the stomach, the multiplication of *R. akari* is exclusively intracellular, and they are evenly distributed over the whole stomach.

Within the cells the position of the rickettsiae is irregular. They are hardly ever found in the portion of the cell toward the lumen as is the case with *R. prowazekii* or *R. mooseri*. Instead they appear beside or more often underneath the nucleus, without invading the nucleus itself. Here they do not form compact aggregations but are either scattered or assembled in small groups in the somewhat diffuse plasma. When the infection partially destroys the intercellular membranes, the rickettsiae tend to form confluent masses of more than usual

density above the basal membrane, and since almost all the cells of the stomach are eventually affected, the number of rickettsiae becomes very great. This is seen more clearly in the smears than in the sections. Vacuolization takes place in later stages and in thick smears the vacuoles may appear to contain rickettsiae, but on section are found to be practically empty (Fig. 5). In exceptional cases,

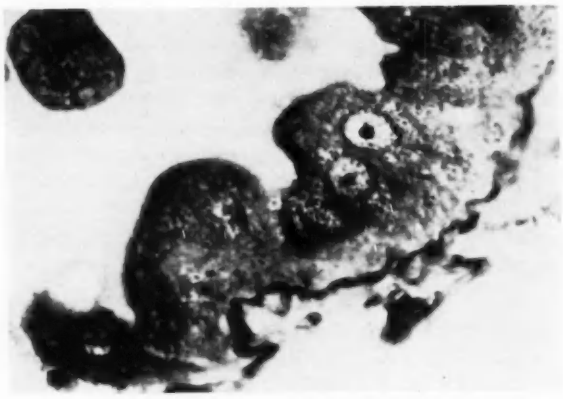

FIG. 4. Stomach section of a louse rectally infected with *R. akari*. 8th day after inoculation. The affected cells are still intact.

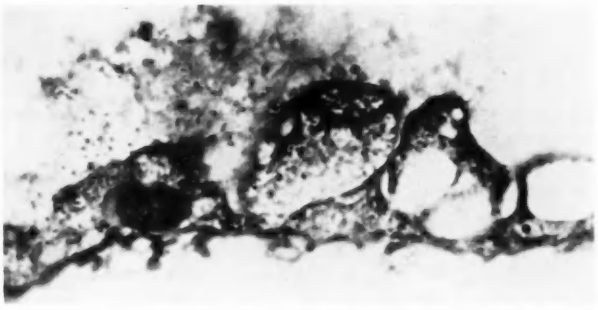

FIG. 5. Stomach section of a louse, rectally infected with *R. akari*. Vacuolization and disintegration of epithelial cells.

the intense vacuolization may cause some cells to protrude into the lumen (Fig. 6). Such infections which at the outset seem fairly harmless have, however, a definite effect on the integrity of the stomach cells and in some cases on the longevity of the lice. The cells eventually dissolve and are liquified, beginning at the base and ending with partial destruction of the epithelium (Figs. 5 and 7). In the early period of infection a few rickettsiae probably enter the lumen of the midgut but are seldom found in the feces at this time. When degeneration of the

cells takes place, free rickettsiae, single or in groups, are found in the lumen attached to fragments of cells, and it is no longer difficult to find them in the feces, although this is somewhat a matter of chance and is therefore irregular.

FIG. 6. Stomach section of a louse rectally infected with *R. akari*. 8th day after inoculation. A vacuolated cell protruding into the lumen of the stomach.

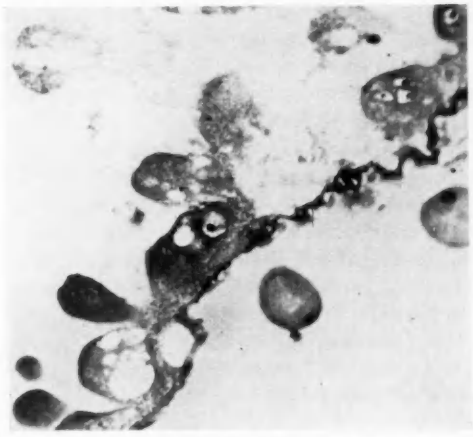

FIG. 7. Stomach section of a louse rectally infected with *R. akari*. 7th day after inoculation. Partial destruction and separation of the affected cells. Magnified 500 times.

This partial destruction of epithelium (Fig. 7) and its sequelae are unlike the phenomena occurring after infection of the louse with typhus fever rickettsiae. In the latter case, 6 to 10 days after infection, depending on the number of rickettsiae and the intensity of the multiplication, a process of destruction begins which affects almost the whole epithelium. With the rickettsiae concen-

trated in the apical pole above the nucleus in the direction of the intestinal lumen, the cells glutted with rickettsiae and blown up under the pressure pour out enormous quantities of the organisms into the lumen and perish in the act. Since the destroyed cells cannot be replaced, the stomach can no longer fulfil its physiological function. The semipermeability fails and the animals are fatally damaged. In spite of this they usually continue to feed but the blood cannot be digested. It stays in the stomach and hemoglobin enters the coelom through the damaged stomach walls. Such lice have a typical light red color which may involve only the stomach or more commonly the whole body, while the stomach of a normal louse takes on a dark red color immediately after feeding.

The behavior of the lice infected with *R. akari* is quite different. The localized loss of some intestinal cells does not produce serious consequences. The stomach continues to function and the life-span is not necessarily shortened or the vitality impaired. Feeding and reproduction take place normally. While an infection with *R. prowazekii* or *R. mooseri* is strictly limited to the stomach and does not cross this border except in some cases immediately before death, *R. akari* occasionally pass from the stomach into the coelom at an early stage. The entry is obviously through thin parts of the wall where cells have perished. Thus in a number of the infected lice one finds the rickettsiae both in the cells of the stomach and in the coelom, though seldom earlier than the 10th day, while in other lice the barrier between intestine and coelom is not crossed.

In the coelom they continue to multiply. Smears from such lice resemble those of animals which have been intracoelomically infected. In the latter, multiplication of the rickettsiae is confined to the coelom, and they do not enter the cells of the ovary as occasionally happens after artificial intracolomic infection with typhus fever rickettsiae (Weyer 1950). Other organs are not infected, except that the stomach cells are in some rare cases infected through the basal membrane. In the coelom the rickettsiae do not depend on cells for multiplication, which takes place extracellularly. Frequently one sees concentrations of rickettsiae in or on blood cells (Figs. 2 and 3), a phenomenon which can also be observed in other species of rickettsiae growing in the coelom.

More serious damage occurs if the rickettsiae in the coelom multiply in large quantities; then the lice die. The damage is probably the result either of the blocking of the blood cells or of the effects of toxins. This makes the intestine permeable although the epithelium may still be almost intact. In addition some stomach cells infected with rickettsiae from the coelom may be destroyed, a condition which manifests itself in a relatively dark reddish color of the lice which always covers the whole body. Such "red" lice live only for a few hours. In some cases the lice may die without showing the red color, although the abdomen of such animals is usually swollen. Rickettsiae entering the coelom from the intestine after rectal inoculation seem to multiply less rapidly and the lice live longer. In this case host and parasite apparently achieve a degree of mutual adaptation.

Virulence tests in the mouse. To test the virulence of *R. akari* after passage through the louse, one series of mice was injected intraperitoneally with coelomic liquid of infected lice, and another with the ground stomachs of lice of the

third and sixth passage. Feces of lice from the same lots were collected from the 10th to the 13th day after inoculation, diluted in broth and injected into mice intraperitoneally. In all cases the mice developed symptoms of disease on or after the 5th day and revealed typical pathological changes on autopsy. Smears from the organs showed rickettsiae and suspensions of mouse spleen and liver tissues transmitted the infection to other mice and to the yolk sacs of eggs. It was evident that the passage of the rickettsiae through the louse had not altered their virulence and pathogenicity for the mouse.

DISCUSSION

The body louse according to the experiments described above is a suitable and susceptible host for *R. akari*. These rickettsiae multiply undisturbed in the louse but their development is limited to the cells of the midgut and to the coelom. Other organs are not affected, and in this respect they behave practically in the same way as *R. prowazekii* or *R. mooseri*. The difference is that *R. akari* are not as numerous as *R. prowazekii*, elect a different location within the stomach cell and do not destroy the whole epithelium. They are found only irregularly in the lumen of the gut and in the feces. However the partial detachment of the cells permits the rickettsiae occasionally to enter the coelom at an early stage, which does not occur with *R. prowazekii*. This kills the lice prematurely, but unless this occurs, rectal inoculation need not shorten the life of the louse in spite of active multiplication of the rickettsiae.

R. akari can be maintained on lice by rectal infection in serial passages and their virulence and pathogenicity are not diminished in the process as may be seen from the mouse virulence tests. The feces of rectally infected lice also contain virulent rickettsiae. However, the feeding of the infected lice on man and their handling has caused no infections in our laboratory. For various reasons only artificial infections of the lice were obtained in our experiments but there is no doubt in my mind that the lice could become infected in the natural way by feeding. This was shown by the infection of the lice with fresh blood from an intraperitoneally inoculated mouse. The blood was diluted with broth so that undoubtedly less blood and fewer rickettsiae entered the stomach than after natural feeding. Nevertheless these lots of lice developed rickettsial infections in the same intensity and within the same time as after artificial infection with stomach suspensions or coelomic liquid.

Of special theoretical interest is the question why *R. akari* develops so easily and well in the louse. Is this an old or a new relationship? The question also arises whether the contact of this rickettsia with man was taking place for the first time when rickettsialpox was discovered in New York in 1946. The probable evolution of the host-parasite relationship in human rickettsial diseases has been discussed by Baker (1943) who considers it probable that the development of human rickettsial infections from what were originally arthropod diseases has taken place in steps. First the arthropod disease is transmitted to the animal which is its primary host; then man enters this arthropod-animal cycle frequently enough to make the disease endemic in human beings; eventually a

second arthropod, always a close associate of man, acquires the infection and a reciprocal transmission results which may become highly specialized and epidemic in man. Thus in typhus fever the primary vector, the flea, infects the rat and transmits the rickettsial organism sporadically to man (murine typhus); man, the secondary host, infects the louse and after a period of adaptation, virulent epidemic typhus results.

According to this hypothetical scheme, Miller (cited by Rose 1948) considers rickettsialpox as a second step. The rodent mite, *Allodermanyssus sanguineus* is the primary arthropod and vector, the mouse the primary host; the human being represents a secondary host.

"Further studies may reveal secondary arthropod vectors. If such vectors, for example the louse, are closely associated with man, the disease may move into its subsequent stages of the epidemic cycle." (Miller).

But is the body louse really the secondary arthropod host? From the relatively harmonious coexistence of *R. akari* and louse, which in our experiments permitted the survival and multiplication of the rickettsiae without materially affecting the host, one might conclude that this contact was not taking place for the first time, but had occurred at a much earlier period so that *R. akari* was already adapted to the louse.

On the other hand it is improbable that a disease like rickettsialpox with such striking symptoms should have remained unrecognized for so long. It is much more probable that *R. akari* is at least an old parasite of the mite and the mouse, and has now for the first time contacted human beings and, in our experiments, the louse. If this agent can exist both in man and in the body louse at the first contact, this may be ascribed to the fact that *R. akari* is closely related to other rickettsiae, which are already thoroughly adapted to the human being and to the louse or other vectors. In fact the complement fixation test shows that in antigenic structure there is a great similarity between *R. akari* and *R. rickettsii* and that there is a relationship between *R. akari* and *R. mooseri*.

The capacity to multiply in arthropods is a characteristic of all "genuine" rickettsiae and is a fundamental element of the definition of rickettsiae. Arthropods, probably ticks, were apparently the first hosts of rickettsiae, which have retained or acquired the ability to multiply also in other bloodsucking arthropods. The louse is an especially favorable host. We know that *R. prowazekii* and *R. mooseri* can probably develop in all bloodsucking lice, as Mooser, Varela and Pilz pointed out as early as 1934. Only recently I have shown that the crab louse, *Phthirius pubis* L. and the hog louse, *Haematopinus suis* L. are also suitable hosts for *R. prowazekii* (Weyer 1951, 1952).

Experiments of my own (Weyer 1952a, and others unpublished) have revealed that also *R. rickettsii*, *R. conorii* and even *R. (Coxiella) burnetii* can exist and multiply in the body louse. The capacity of the rickettsiae to multiply in certain tissues and cells is however not always a sign of an old relationship between host and parasite. For instance their growth in the yolk sac of the chick embryo or even in the coelom of the larva of *Tenebrio molitor* L. (Weyer 1950) proves that there are environmental conditions which are, *a priori*, suited to the biological needs of the rickettsiae and to which they do not have to adapt themselves.

To biological thinking, however, it is more plausible to suppose a close relationship between the different species of rickettsiae based on a common origin. This argument has been discussed in greater detail by Mooser (1946) among others. It is not astonishing that, in passing from primary hosts to secondary hosts, from one vector to another, etc., the properties of the rickettsiae or of the rickettsial strains have become changed in the course of time by mutation and selection, thus accounting for the different types of disease. It is not unlikely that when some strains of rickettsiae come into contact with human beings, they might produce new diseases even now. Rickettsialpox may be an example. But ultimately the old properties typical of all rickettsiae reappear under favorable conditions despite minor differences and deviations.

SUMMARY

1. *R. akari* was transmitted rectally and intracoelomically to the body louse by inoculation with peritoneal exudate of mice. In both cases the rickettsiae developed regularly and intensively in the louse. The same result was obtained when the lice were injected rectally with the blood of sick mice.

2. Multiplication of the rickettsiae was always intracellular in the stomach wall, localized particularly in the basal part of the epithelial cell; it was extracellular in the coelom liquid, from which the organisms in rare cases entered the stomach cells as well.

3. Multiplication of the rickettsiae in the stomach cells was moderate and did not deform them or cause the premature death of the louse; in the coelom, however, intense multiplication soon produced damage from which the louse died in a few days.

4. After rectal inoculation the rickettsiae invade and destroy or detach some of the stomach cells. Through the partially damaged walls they may escape into the lumen of the stomach on one side and appear in the feces, or less frequently into the coelom on the other, and by intense multiplication cause the rapid death of the louse.

5. By rectal transmission of ground stomach or coelom liquid of positive lice, *R. akari* can be maintained by serial passage in lice without losing virulence and pathogenicity for the mouse. The feces of the lice also contain virulent rickettsiae.

6. The behavior of *R. akari* in the louse can best be explained by its close relationship to other rickettsiae, if we suppose that the capacity to develop in arthropods is a fundamental characteristic of all rickettsiae and that the different species of rickettsiae all have a common origin.

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EFFECTS OF FEEDING COLOSTRUM CONTAINING *COXIELLA BURNETII* ANTIBODIES TO NEWBORN CALVES OF TWO CATEGORIES

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In the course of studies conducted in southern California for the purpose of ascertaining the presence of specific antibodies to *Coxiella burnetii* in dairy cattle (Huebner *et al.* 1948; Shepard *et al.* 1948), it was noted (with one dubious exception) that sera from more than one hundred newborn calves of serologically negative dams did not become positive at any time during the several months of post natal observation. On the other hand, sera from calves of serologically positive dams usually did become positive even though the calves were bucket fed and, in many instances, did not suckle from their own dams (Huebner *et al.* unpubl.). The source of antibodies in the calves of the latter group was not immediately obvious; however, three possible sources suggest themselves at once: 1) the specific antibodies observed in the calves might represent a passive placental transfer from the dam; 2) the antibodies might be transmitted from parent to offspring through the medium of colostrum; or 3) the antibodies might arise as a result of infection of the calf with *C. burnetii*.

Since in the case of other infections, such as brucellosis (Little *et al.* 1922; McDiarmid 1946) concentration of antibodies in the colostrum of the newly parturient animal and transmission through this medium to the offspring has been demonstrated, the study presented here was designed to ascertain whether complement-fixing antibodies against *C. burnetii* found in dairy cattle are similarly transmitted through the colostrum to the new-born calf.

METHODS

Pregnant cows of two dairies, where infection with the rickettsia was known to be common, were kept under close surveillance as they neared parturition; this was done so that the calves of these dams could be separated from their mothers before suckling had occurred. It was thus possible to obtain new-born calves in two categories:

a) Three calves from dams with titers of *C. burnetii* complement-fixing serum antibody of 1:64.

b) Four calves from dams with titers of complement-fixing serum antibody of less than 1:4. These dams are considered serologically negative. Further, a complement-fixation test was performed on the colostrum from each dam in this group. Specific antibodies were not detected in any instance, providing further evidence that these animals were serologically negative.

Colostrum was obtained from 3 parturient cows from the same herd. Each donor cow was shown to be serologically positive (complement-fixing antibody titers were all greater than 1:32) on 3 separate occasions prior to parturition,

at parturition, and following parturition. The complement-fixing antibody titer of the colostrum fed ranged from 1:64 to 1:256.

Colostrum from the donor cows was obtained by milking into steam-sterilized buckets and was stored in sterile one-gallon glass containers at 38–42°F. The maximum period of storage was 48 hours. During the first 48 hours after birth, the calves in this experiment were fed total amounts of colostrum varying from 0.36 to 3.84 liters. The colostrum was warmed to 100°F. before feeding, and was fed 3 times daily from sterile nipple-capped bottles.

After the 48 hour period of colostrum feeding, all calves were fed pooled whole milk obtained from a string of cows under treatment for other conditions, such as foot-rot and actinobacillosis, and from normal, newly parturient cows. Thirty per cent of the individual samples of milk were shown to contain rickettsiae by inoculation into guinea pigs. None of the calves of negative dams showed evidence of infection at the termination of the observation period (see results).

Complement fixation tests were performed upon blood specimens taken from each dam two months prior to parturition, at parturition, and at intervals following parturition. Complement fixation tests were performed on blood specimens taken from the jugular vein of each calf at birth, and at intervals thereafter. All test were performed at the National Institutes of Health, using the technique regularly employed at that laboratory (Bengston 1941, 1944).

Numerous specimens of blood from both dams and calves, and colostrum from the dams were also examined for the presence of *Coxiella burnetii* by inoculation into guinea pigs as described elsewhere (Huebner *et al.* 1948); in no instance was the organism recovered.

RESULTS

The results obtained from feeding serologically positive colostrum to calves of serologically positive dams are summarized in Table 1, Part I.

It will be seen from the table that serum from dam no. 1 possessed complement-fixing antibodies to *C. burnetii* in a titer of 1:64 throughout the period of observation. At birth, serum from the calf (1A) of this dam was found to have an antibody titer of less than 1:4 (negative). A serum specimen obtained one week postnatally, 5 days after colostrum with an antibody titer of 1:64 had been fed, was found to contain complement-fixing antibodies in a titer of 1:64; this titer remained essentially unchanged at the end of one month.

Similarly, serum from dam no. 2 was shown to contain antibodies (titer 1:64) throughout the experiment. The calf (2A) of this dam possessed serum antibodies in a titer of 1:8 at birth. At the end of one month, following a 48 hour period of feeding with colostrum having an antibody titer of 1:256, serum from this calf possessed antibodies in titer of 1:32.

The third dam also remained serologically positive throughout the 3 months of observations; its calf (3A) was serologically negative at birth. After receiving colostrum with an antibody titer of 1:64 for 1 day this calf possessed serum antibodies of 1:64, and the antibody content of the serum remained essentially unchanged 2 weeks later.

Thus, in summary, calves of the 3 serologically positive dams in this group possessed no, or very little, complement-fixing serum antibody to *C. burnetii* at birth. After ingesting colostrum with an antibody titer ranging from 1:64 to 1:256 however, each calf showed in its serum the presence of specific antibodies which persisted for at least one month.

The results obtained from feeding serologically positive colostrum to calves of serologically negative dams are presented in Table 1, Part II.

TABLE 1

Result of feeding serologically positive colostrum to calves of serologically positive dams, and to calves of serologically negative dams

ANIMAL NUMBER	COMPLEMENT-FIXING ANTIBODY TITER OF SERUM						C.F. ANTIBODY TITER OF COLOSTRUM FED CALVES	AMOUNT OF COLOSTRUM FED CALVES FIRST 48 HOURS
	Two months prepartum	At parturition	Post partum					
			One day	One week	Two weeks	One month		
Part I								
								liters
Dam 1	1:64	1:64				1:64		
Calf 1A		<1:4		1:64		1:32	1:64*	1.44
Dam 2	1:64	1:64				1:64		
Calf 2A		1:8				1:32	1:256†	3.84
Dam 3	1:64	1:64				1:32		
Calf 3A		<1:4	1:64		1:32		1:64‡	0.36
Part II								
Dam 4	<1:4	<1:4		<1:4		<1:4		
Calf 4A		<1:4		<1:4		<1:4	1:64*	1.44
Dam 5	<1:4	<1:4				<1:4		
Calf 5A		<1:4	<1:4			<1:4	1:256†	3.84
Dam 6	<1:4	<1:4				<1:4		
Calf 6A		<1:4	<1:4	<1:4		<1:4	1:64‡	1.56
Dam 7	<1:4	<1:4			<1:4			
Calf 7A		<1:4	<1:4		<1:4		1:64‡	0.54

* Donor serum antibody titer 1:64.
† Donor serum antibody titer 1:64.
‡ Donor serum antibody titer 1:32.

It will be seen from the table that serum from all four dams (nos. 5–8) in this group remained serologically negative throughout the experiment. Moreover, although the 4 calves of these dams were fed serologically positive colostrum from the same source and in the same manner as described for the preceding group, at no time were complement-fixing antibodies demonstrable in the sera of these calves. Thus in summary, it was not possible in this series to evince measurable complement-fixing antibodies in serologically negative calves of serologically negative dams by the ingestion of serologically positive colostrum alone.

DISCUSSION

A number of studies (Little *et al.* 1922; Famulener 1912; Smith 1923; McDiarmid 1946; Young *et al.* 1950) have suggested that in some species of animals agglutinating, hemagglutination-inhibiting, and neutralizing antibodies may be passively transmitted from the parent to offspring through the medium of the antibody-containing colostrum. In most of these investigations, it should be pointed out, no consideration is given to the antibody status of the parent; data concerning the immune status of the animals have been limited to the offspring. In other studies, the offspring have been fed the colostrum from their dams, irrespective of their serological status.

In the experiments reported here, colostrum containing antibodies was obtained from dams not otherwise used in the study; this colostrum was fed to calves in which the serologic status of both parent and offspring had been established. When no antibodies were demonstrable in the serum of the dam, it was not possible to detect antibodies in the serum of the calf before or after feeding of colostrum containing antibodies in high titer. On the other hand, when the dam was shown to possess serum antibodies, the feeding of antibody-containing colostrum was in each instance followed by the appearance of antibodies in the serum of the offspring. It does not seem likely, therefore, that complement-fixing antibodies are carried to the offspring through the medium of colostrum alone; this is contrary to the usual concepts.

Also, at least in this particular series of experimental observations, it appears that antibodies in amounts demonstrable by complement fixation test usually were not transferred to the calf across the placental membranes, since sera of the calves taken prior to suckling were shown to have no demonstrable antibodies, or at the most, antibodies in very low titers (1:8). The possibility exists, of course, that antibody in small, undetectable amounts was transferred transplacentally, and was brought to the threshold of demonstrability by the addition of antibody contained in the ingested colostrum. The possibility of "blocking antibodies", present in the calf sera at birth, has not been eliminated, however.

In searching for the source of antibodies in calves nos. 1–3 of this series, therefore, the possibility remains that the antibodies demonstrated arose as a result of infection. Conceivably, such infection may have arisen from the ingestion of infected milk during the period following that in which colostrum was fed. Since all 7 experimental animals were fed milk from the same pooled source, however, without evidence of infection in 4 calves from negative dams, infection in this manner does not appear probable. Furthermore, an additional 75 calves from serologically negative dams failed to develop antibodies although fed on infectious milk, giving further evidence that infection arising from ingestion is an uncommon event (Huebner *et al.* unpbl.)

Congenital or intrauterine infection of calves nos. 1–3 appears to be a distinct possibility, since it has been demonstrated that the placentas of cattle infected with *C. burnetii* may contain large numbers of the rickettsia (Luoto *et al.* 1950). Also, placentas from naturally infected and artificially infected sheep have been shown to contain *C. burnetii* in high concentration (Welsh *et al.* 1951; Winn

et al. 1951). It should be noted, that the rise in antibody titer during the first day of life observed in calf no. 3A would be difficult to explain on the basis of infection acquired at or after birth.

Whether congenital or intrauterine infection is a common event, thereby giving rise to specific antibodies in new-born animals, will not be clearly defined, however, until more extensive experimental observations have been made.

SUMMARY

Colostrum containing complement-fixing antibodies against *Coxiella burnetii* was fed to new-born calves in two categories:

a) calves, which aad not suckled, from serologically-positive dams, and

b) calves, which had not suckled, from serologically-negative dams.

Calves from serologically-negative dams did not develop specific antibodies over a period of one month following birth, whereas calves from serologically-positive dams developed specific antibodies during this period.

The possible source of such specific antibodies is discussed.

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ENDEMIC TREPONEMATOSIS (BALASH OR BEJEL) IN SAUDI ARABIA

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INTRODUCTION

While reservoirs of endemic treponematosis have been reported from several countries of the Middle East (4–20), no known survey has been made in Saudi Arabia. In Syria and Iraq, bejel has been described by Hudson (1928–1939); in Palestine as "firjal" by MacQueen (1934), and in Trans-Jordan by Hasselman (1938).

Since Saudi Arabia constitutes four-fifths of the Arabian Peninsula, a survey seemed indicated. The nomadic tribes of the Nejed district, through which passes the Trans-Arabian Pipe-line, are composed, in part, of Saleebies who are Christian converts of the Muslem majority. They migrate freely across national boundaries throughout the Middle East.

Physicians of the Trans-Arabian Pipe-line medical organization had observed a relatively high prevalence of treponematosis among Bedouins of the Nejed district. It is called "balash" locally, meaning the "catastrophe" or "trouble." In the district of Jejaz (to the west) the disease is known as syphilis or "frandji"; the latter term is used in several Arab countries and in Turkey, and means "the foreigner."

Because of the increasing strategic importance of Saudi Arabia we obtained permission from the Saudi Arabian Government to study treponematosis among their tribal people. The full cooperation and facilities of the medical department of the Trans-Arabian Pipe-line organization were provided. The station hospital and its staff at Badana, about 600 kilometers from the coast of Lebanon, were put at our disposal.[2] The Bedouin tent dwellers in the area, many of whom belonged to the Saleeby tribe, came to the station hospital for the survey. These people live under primitive conditions, crowded together in tents, in utter misery and in a very poor hygienic environment.

LABORATORY FINDINGS

Our purpose was to examine natives, many of whom had had no previous medical care. During May 1951, 82 unselected tribesmen were examined, gave fragmentary histories and provided blood for serologic tests. All specimens were sent by plane to the American University in Beirut, Lebanon where Kolmer complement fixation, V.D.R.L. flocculation and Kahn standard tests were performed on each. One specimen was anticomplementary.[3]

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[2] We are indebted to Dr. Frank J. Zukoski, Medical Director, Trans-Arabian Pipe-line Co., for his many courtesies during this study.

[3] We are indebted to Dr. K. S. Krikorian for the serologic tests.

Of the 81 blood samples, 30 or 37.8% were positive by two or more test procedures. Twenty-five, or 83% of the tribesmen in this group gave a history of balash. Of the remaining patients examined, only six gave a history of balash. These had negative serology and no physical evidence of the disease. On the other hand only ten, or one-third of the patients with positive serology had physical evidence of endemic treponematosis.

One female child of eleven years had papular-erosive mucous patches on the tongue, lips and tonsils, with marked enlargement of the left cervical lymphatics, and one node which may have been a satellite bubo. Dark field examination revealed the presence of *Treponema pallidum* which was indistinguishable from that of syphilis. The serologic tests were strongly positive in this patient.

CLINICAL MANIFESTATIONS

There were approximately 4% in the age group 1 to 9 years; 22% in the age group 10 to 19; 30% in the age group 20 to 29; 20% in the age group 30 to 39; 15% in the age group 40 or older. The remainder were not certain of their ages. The ratio of males to females examined was approximately three to one. Of the tribes represented, so far as could be determined, the majority were Saleeby, next Rawaly, then Shamary, Hazimi and Yameny. Others, especially among the soldiers, did not reveal their tribe. However, all were native to Saudi Arabia, since no foreigners were included in the study. An attempt was made to obtain an unselected group of tribes people representative of the area studied.

The majority of cases had acquired balash in childhood and could be considered nonvenereal. We believe, however, that venereal treponematosis among adults did occur. Gonorrhea is not a rare disease among Bedouins and hetero- and homosexuality are known to exist. Spermatozoa were demonstrated in the stool specimens of an adult male. Because the majority of infections observed were acquired in childhood, the disease is less likely to be in the infectious stage when sexual maturity occurs. This has been given as one reason for its nonvenereal classification.

In three patients there were juxta-articular nodules on elbows, knees and ankles. These have been previously described by Hudson in bejel but are a rare finding among patients with syphilis in Lebanon. A nodule was removed and examined by Dr. Philip Sahyun, Professor of Pathology. The description was similar to that given by Weidman for a similar nodule removed by Hudson. It was: "Fibrous and showed a moderate degree of diffuse leukocytic infiltration. There were foci of granulation tissue in poorly arranged tubercle formations. These consisted of large mononuclears, a few lymphocytes, plasma cells and rare eosinophils. Giant cells were observed also. There was no caseation. Acid-fast staining revealed no mycobacteria. The picture was that of "granulating inflammation." See photomicrograph, Fig. 1.

Two patients had gummata of cutaneous tissues. Two had periosteitis and osteomyelitis. Two had a generalized lymphadenopathy. One male patient aged 18 years had a systolic murmur in the aortic region.

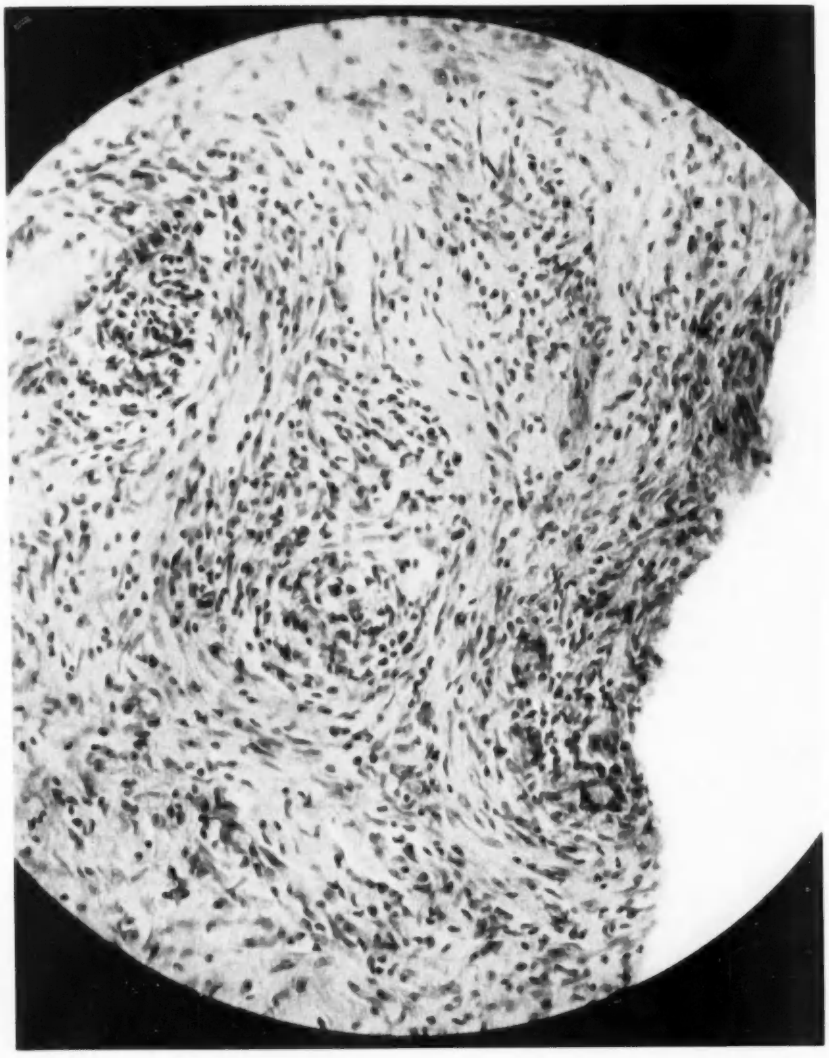

FIG. 1. Juxta-articular nodule removed from the elbow of a patient with treponematosis (×285).

Eight of the patients examined were below the age of twelve years, and five of these had positive serology. Congenital lesions were not observed. No genital lesions nor scars were demonstrated in any patient.

SUMMARY

1. The prevalence of treponematosis among the tribesmen around Badana, Saudi Arabia is 37.8% out of a sample of 81 unselected cases.

2. On the whole, the clinical picture is similar to syphilis and bejel as reported by Hudson, Hasselman, Akrawi and seen by one of us (H. T. C.) in the Deir ez Zor district.

3. The majority of cases had acquired the disease in childhood, but some later in life.

4. Possibly the majority had acquired it nonvenereally, but venereal contacts could not be disregarded.

5. It is realized that the group screened is small and statistical error is likely. However, the prevalence of treponematosis is high.

6. Saudi Arabia constitutes a reservoir of infection with possibility of transfer to other areas.

7. The clinical picture, incidence and laboratory findings paralleled Hudson's study of similar tribes in Iraq (1951).

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STUDIES ON SCHISTOSOMIASIS

VI. Control of the Snail Host of Schistosomiasis in Japan with Sodium Pentachlorophenate (Santobrite)

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World War II stimulated an interest in schistosomiasis and considerable work was carried out on the biology of the known and potential snail hosts of the three human schistosomes. In addition, emphasis was placed upon investigations on methods for controlling the various snail hosts, largely through the use of molluscacides (Stirewalt and Kuntz 1947; 1947a). Two types of snail host exist, aquatic and amphibious. The intermediate hosts of schistosomiasis japonica are amphibious snails, and their control constitutes a difficult problem. In 1945 field laboratory screening and plot tests were undertaken in the Philippines by such groups as the Commission on Schistosomiasis (McMullen and Graham, 1947), followed by more extensive laboratory and field screening tests mostly against aquatic snails (Nolan and Berry, 1949; Berry, Nolan and González, 1950; Kuntz and Stirewalt, 1950; Jachowski and Stirewalt, 1950; Kuntz and Wells, 1951). Ardel Azim and Barlow, beginning in 1943, carried on extensive field work in Egypt on the control of schistosomiasis snails. The results of this program were summarized by Abdel Azim in 1949. Pesigan and Masilungam (1950) have worked with field plots on the problem of controlling *Oncomelania quadrasi*, the amphibious snail host of *Schistosoma japonicum* in the Philippines.

The plan which was evolved by the Department of Medical Zoology of the 406th Medical General Laboratory for the development of an effective molluscacide to control schistosomiasis japonica included the following steps: (1) the laboratory screening of potential molluscacides; (2) the testing in the field, i.e., plot tests of the more promising chemicals found in (1); (3) the attempt to control the snail host of schistosomiasis japonica in a large area over a period of several years through the repeated application of one of the best chemicals found in (1) and (2) above; (4), if (3) were successful, the final step involved the attempt to control the snails in one of the five known endemic areas of schistosomiasis in Japan with the same chemical used in (3) above.

The first two steps in this program were undertaken in 1947 by McMullen *et al.* of the Department of Medical Zoology in cooperation with the National

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Institute of Health and the Yamanashi Medical Research Institute of Japan. This study yielded three promising chemicals; sodium pentachlorophenate (Santobrite[6]), dinitro-o-cyclohexylphenol (DN-I) and the dicyclohexylamine salt of dinitro-o-cyclohexylphenol (DN-111 or K-604) (1948, 1949 Ann. Hist. Repts.; McMullen, Ishii and Mitoma, 1948; McMullen *et al.* 1951, 1951a). Meanwhile Japanese workers continued the search (Miyaki, Ikeda, Ishii and Mitoma, 1950). A fourth chemical, calcium cyanamide, had been shown to be quite effective by the Japanese and had been used by them for some years, but it never satisfactorily controlled the snail host, *Oncomelania nosophora* (Ono, 1949).

Later McMullen (1951, 1951a) extended the field plot tests to show the lethal effect of single applications of sodium pentachlorophenate and the dinitro compounds (*vide supra*). Fall and spring applications were made in some cases but in most instances a combination of different chemicals was used; only about 1000 feet of ditch were given successive applications of Santobrite (HSPA Activator) and here the results were not clear cut as evidence of repopulation was noted. Thus the need for initiating the third stage of the program was apparent. This was planned in 1949 and the project, known as "Operation Santobrite",[7] was begun in the Spring of 1950 (Hunter, Ritchie *et al.*, 1951). This project was of sufficient magnitude to serve as a basis for determining whether molluscacides could be made effective and practical for a control program of one of the endemic areas in Japan.

MATERIALS AND METHODS

The area. Nagatoishi-cho in Fukuoka Prefecture on the island of Kyushu, Japan, was selected for this project. The area which supported a population of 1050 persons consisted of 150 acres enclosed by dikes located in the heart of the schistosomiasis belt. This community was selected for the study because of the abundance of snails and the high incidence of schistosomiasis in the area; furthermore, the people and fields were relatively isolated from the surrounding area by the dikes and consequently there was less danger of introducing infected snails from outside sources (Fig. 1). The people of the area were 73% infected, both in 1948 and 1950. Since 1948, treatments had somewhat reduced the intensity of the infections in the human population, although the incidence remained the same and the snails continued to be quite heavily infected.

Procedure. It was decided to apply the chemical biannually, in the spring and the fall. After consultation with statisticians, a total of 170 quadrats were selected at random and marked by stakes. All snails were collected from each quadrat, counted, and the total number of living snails determined. Upon com-

[6] In order to avoid confusion in the literature it should be pointed out that the sodium pentachlorophenate as supplied by The Monsanto Chemical Co. goes under the trade name of "Santobrite" rather than "HSPA activator". McMullen *et al.* 1951 used the former and later (1951a) used "HSPA Activator" for the same chemical. Since the manufacturers use the term "Santobrite" in their literature, we will also use it in this paper.

[7] This project was made possible by The Monsanto Chemical Company which generously furnished a ton of sodium pentachlorophenate (Santobrite) for this control project in Japan.

pletion of these pretreatment counts the molluscacide was applied and two weeks later post-treatment counts of living snails were made in adjacent quadrats. The per cent reduction in snail population was then calculated. During the fall 20 new quadrats were added. These were located beyond the ditches, usually at the edge of the rice paddies. Each time a pre- or post-treatment count was made,

FIG. 1. Sketch map of the Nagatoishi and control areas.

care was taken not to re-examine a quadrat which had been previously studied.

Controls. Two types of controls were used. The first consisted of adjacent untreated areas where snails were collected when the counts were made. Initially, 12 control quadrats located in neighboring paddies beyond the dikes were selected and the second year another 12 were added in the river bottom near the inlet of the main irrigation ditch. This enabled us to determine whether there had been

an excessive reduction in snails in nature in untreated areas during the period of the experiment. The second type of control was based upon information on the number of new infections over the same period of years, which occurred annually, prior and subsequent to treatment, in the experimental area, as well as data on existing infections in a neighboring, nontreated control area.

Method of application. Voluntary labor for six crews was furnished by the local Japanese. Each team consisted of a supervisor from the 406th MGL or NIH, a foreman, interpreter, chemical mixer, at least four water carriers, ten sprayers, and five grass cutters. Each team was supplied with six sprinkling cans and four pressure sprayers (two Lofstrand and two of local manufacture). The latter were used to reach under culverts, overhanging banks, and into rodent or other burrows. In addition to the sides of the ditches, the water in the ditches and the tops of the banks were also sprayed. Following the initial treatment, the edges of the rice paddies were subsequently included in the spraying. However, as snails were rarely encountered in the paddies, it was not necessary to spray the flooded paddy. When they did occur, they were always near the inlet to the paddy and so would usually be caught when the margins next to the ditches were treated. Sodium pentachlorophenate (Santobrite, 390 gms.) was dissolved in about 20 gallons of water, giving a dilution of approximately 1:200, and was applied at the rate of approximately 390 mg. per square foot. The length of ditch sprayed varied between 150 and 200 feet depending upon the width of the ditch.

RESULTS

The first year (1950). The initial pretreatment collection made in March 1950 yielded a total of 2436 *Oncomelania nosophora*, of which 2364, or 97 per cent were viable. This figure of 2364 viable snails constituted the base line for further comparisons. Santobrite was then applied and when the post-treatment collections were made about two weeks later in early April, only 43, or 3 per cent, of the 1433 snails that were collected were alive. This represented a population reduction of 98.14 per cent of the viable snails (Table 1). The counts of the untreated controls revealed an increase of dead snails of 9 per cent when the post-treatment counts were compared with the pretreatment ones (Table 1). The increase in the ratio of dead to living snails in the treated area, as well as the overall reduction of population, was highly significant when compared with that of the controls.

In checking the effectiveness of the spraying in the spring, a number of additional quadrats were examined which had not been treated. Most of these new quadrats were near the edges of, or under, piles of straw at the margins of the paddies. Since some viable snails were encountered, it was decided in the future to spray the edges of the rice paddies and add 20 new quadrats to cover such areas.

The pretreatment counts were made in early October of 1950, at which time a total of 568 viable snails were recovered. This was a marked increase over the 43 living snails found at the time of the spring post-treatment count. The ditches and edges of the paddies were then sprayed with Santobrite. When the post-

treatment counts were made in early November 1950, 234 viable snails were recovered, which represented a 90.2 per cent reduction in the viable snail population over the original pretreatment collection figures (Table 1).

The second year (1951). The pretreatment counts in the spring of 1951 of the 190 quadrats yielded only 32 viable snails which represented 98.6 per cent control when compared with the initial pretreatment counts made at the beginning of the experiments. Following treatment, only 13 living snails were found, representing a reduction of 99.5 per cent in the snail population (Table 1).

TABLE 1

Summary of the results in the attempt to control Oncomelania nosophora *in Nagatoishi-cho, an area endemic for schistosomiasis, during 1950 and 1951*

Treated Area

PERIOD	NO. OF QUADRATS	PRETREATMENT COUNTS				POST-TREATMENT COUNTS			
		No. alive	No. dead	Total	% reduction in living snails	No. alive	No. dead	Total	% reduction in living snails*
Spring '50	170	2364	72	2436	3.04†	43	1390	1433	98.14
Fall '50	190‡	568	34	602	76.0	234	432	666	90.2
Spring '50	190	32	137	169	98.6	13	306	319	99.5
Fall '51	190	115	12	127	95.1	24	65	89	99.01

Controls

PERIOD	NO. OF QUADRATS	NO. ALIVE	NO. DEAD	TOTAL	% DEAD	NO. ALIVE	NO. DEAD	TOTAL	% DEAD
Spring '50	12	80	2	82	2.4	62	8	70	11.4
Fall '50	12	109	13	122	10.6	97	1	98	1.0
Spring '51	24	339	11	350	3.1	345	4	349	1.1
Fall '51	24	267	106	373	28.4§	293	18	311	5.8§

* Based on the living snails in the first pretreatment counts.

† Percentage of dead snails in the initial pretreatment collection.

‡ Included 20 new quadrats along the edges of paddies.

§ One-half of the controls had been inadvertently treated with calcium cyanamide.

However, in the fall a total of 115 viable *O. nosophora* was encountered prior to treatment. This figure dropped the control attained from 99.5 per cent in the spring to 95.1 per cent before the area was sprayed. Following the last spraying for 1951, 24 of the 65 *O. nosphora* that were recovered were alive, thus raising the control achieved to 99 per cent (Table 1).

Controls: (1) Snails. It will be seen from Table 1 that not more than 11.4 per cent of the *O. nosophora* collected from the control quadrats were dead except in the fall of 1951 where calcium cyanamide had been applied during the summer. Usually the percentage of dead snails was below 5 per cent. Further reference to this table reveals that this is well below the percentage of dead snails found following treatment with the molluscacide in Nagatoishi (Table 1).

(*2*) *New cases of schistosomiasis.* Since schistosomiasis is not a reportable disease in Japan, the data presented here must be considered as only an approximation. However, attempts were made to secure as accurate information as possible and consequently these data may be regarded as another control.

The best criterion of the effectiveness of the snail control achieved is the number of new cases of schistosomiasis occurring each year in Nagatoishi, compared with a control community of approximately equal population. Prior to the initiation of the control program, 25–35 new cases were reported from Nagatoishi each year. During 1950, the first year of treatment, only 5 were known to have acquired the disease while in 1951, none were reported. During this same period, the number of new cases in the control village remained constant, i.e. 20–30. While such figures must be interpreted with caution, they are highly suggestive.

DISCUSSION

Area treated. As previously indicated, this paper constitutes the third phase of the attempt to control the snail host of *S. japonicum* in Japan. McMullen *et al.* (1951a), who were actually working on the second stage of the program in the Kofu area, treated approximately 18 miles of ditches characterized by different types of environments. It should be emphasized that the Kofu work covered the field testing of a variety of chemicals and that, although some areas were treated more than once, only about 1000 feet of ditch were given two applications of sodium pentachlorophenate. Consequently the Nagatoishi area on Kyushu, consisting of 150 acres with its 12 miles of ditches, which were treated four times with sodium pentachlorophenate (spring and fall for two years), constitutes the first major test of the control of *amphibious* snails. A preliminary report of the first year was made previously (Hunter *et al.* 1951). In the meantime various groups such as Abdel Azim and Barlow (1946) and Kuntz and Wells (1951) in Egypt and the workers from the National Institutes of Health under Dr. Wright (Nolan and Berry, 1949; Berry, Nolan and González, 1950) have made extensive field control studies on the *aquatic* snail hosts of schistosomiasis. Thus it appears that extended field trials are being conducted on both types of the schistosome snail hosts throughout the world.

Types of ditches treated. It should be pointed out that the 12 miles of ditches in Nagatoishi covered a wide variety of ditch environments. For example, a portion of the main inlet ditch was lined with concrete (Fig. 2). When the pump was operated the flow was very rapid as water was being pumped into this ditch at over 800 cu. ft. per minute. This main ditch was over a yard in width and depth. The primary ditches (Fig. 1, A–H) varied from dirt lined to rock walled and from two feet to nearly ten feet in width (Figs. 3–5). The numbered ditches (with the exception of "O" and "I") were so narrow as to be readily stepped across and were never more than two or three feet in depth (Figs. 6–7). In addition to these environments, ditch "O" expanded at intervals to form ponds (Fig. 8); in other areas isolated "fish ponds" were treated. From this description it can be seen that a wide variety of habitats was actually treated which resulted in a comprehensive test of the molluscacide. In addition to the actual ditch that was treated,

it must be remembered that the entire area from paddy to paddy was sprayed. In the case of the lettered ditches this always included an ox cart path or the equivalent. In this way considerable dry, hard soil was sprayed. It is virtually certain that these paths actually did not provide a habitat suitable for the snails and in an extended control program such areas could be disregarded with a considerable saving of chemical.

Effects upon plants and animals. Sodium pentachlorophenate (Santobrite) kills many of the dicotyledonous plants, but fortunately has little effect upon the monocotyledons in the dilution of chemical used. There was no evidence of

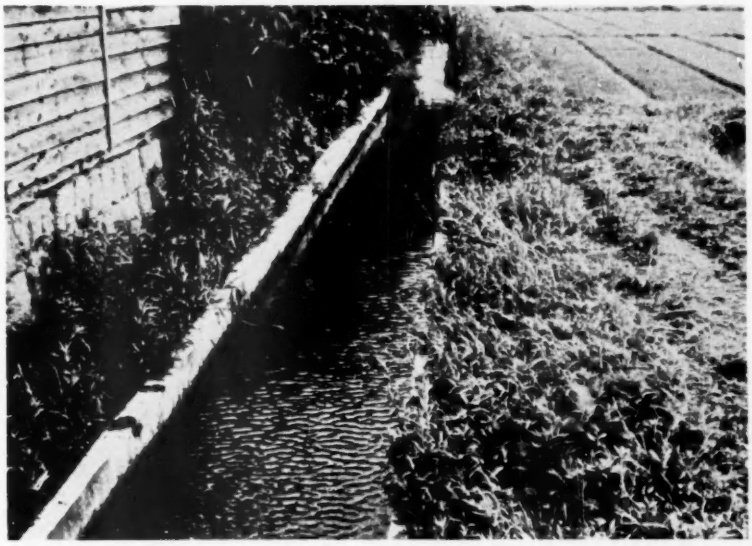

FIG. 2. Main inlet ditch; when the pump is running, water enters this at the rate of 800 cu. ft. per minute. (See Fig. 1).

harm to young or mature wheat or rice plants due to the Santobrite applied on the paddy edges or the stalks of grain. On the other hand, fish, newts, frogs, leeches, and snails, including the large snail *Vivipara japonica*, were rapidly killed. However, the aquatic fauna returned to normal within 4–6 weeks.

Toxicity studies. In addition to the studies made by the manufacturers of sodium pentachlorophenate, the Department of Chemistry of the 406th Medical General Laboratory carried out a series of additional tests to determine the toxicity to mammals. It was ascertained that Santobrite was not fatal to mice when administered in a concentration of 1 per cent over a period of 20 days. No evidence of pathologic changes was noted at autopsy or when the organs were sectioned. This was interpreted to mean that no danger existed to man or domestic animals in the dilution used in the application of the chemical, as both

FIG. 3. One of the lettered ditches. Grass cutters are preparing the ditch for spraying. Such ditches are treated from paddy to paddy.

FIG. 4. Spraying a stone-lined ditch with pressure sprayer. Snails were found originally in the crevices between the stones.

FIG. 5. Pressure sprayers were used to reach undercut banks, rodent burrows and the like.

FIG. 6. Spraying with Santobrite along one of the numbered ditches.

FIG. 7. One of the numbered ditches showing the vegetation prior to its being cut.

FIG. 8. Ditch "O" with dike in background and the end of one of the ponds formed along this ditch (See Fig. 1).

the concentration and length of dosage were greater than would be encountered in the field. None of the cattle in the experimental area were affected. Recently Stohlman (1951) and Herdt *et al.* (1951) reported the LD 50 in mg./kg. in rats for Santobrite was 100. While this was higher than for some of the other chemicals tested, it indicates a considerable margin of safety.

Residual effect. Reference to Table 1 shows that the post-treatment count made in the fall of 1950 yielded 234 viable snails. However, when the pretreatment counts were made the following spring, there were only 32 living snails recovered. This suggests that the sodium pentachlorophenate affected the snails either by weakening them or by poisoning them upon contact or upon ingestion of treated soil or vegetation. Similar results are anticipated in early 1952.[8] No significant decrease was noted in the controls.

Summer increase in snails. Several possible explanations are apparent which may account for the increase in snails between the spring and fall application of the chemical. First it should be borne in mind that this increase could not have been due to the additional 20 quadrats, as only 33 viable snails were taken initially from these areas. It seems more likely that either (1) all of the snails were not out of hibernation at the time of the post-treatment counts in April; or (2) new snails were introduced from the river outside of the dikes; or (3) the increase represented the progeny of the survivors.

The first possibility can be ruled out since the spring spraying was delayed at least two weeks in the second year (spring of 1951) and there still was an increase in snails during the summer.

The second hypothesis depends upon the possibility that new snails were introduced through the pump which sucked the water from a canal originating in the Chikugo River. This seemed likely since colonies of snails were known to exist along the river bottom on the upstream side of the intake canal which was 150 yards in length. In order to check this possibility, screens were built which could be placed across the main concrete irrigation ditch inside the dikes in Nagatoishi near the outlet of the pump. By checking the rate of flow of the pump and the length of time the screens were in place, it was possible to compute the number of cubic feet of water that were strained.

Using this technique the pump was checked when it was first turned on in the spring of 1951. Local records revealed that the Chikugo River often floods during the heavy rains that normally fall during the month of July. At such times the entire river bed between the dikes is under water and it seemed possible that snails from the upstream colonies which became inundated might settle into the inlet canal as the flood waters commenced to recede and so be pumped into Nagatoishi when the pumps were started again. This was checked (1) when the pumps were started after the flood (97,200 cu. ft.); (2) when the water was down to the level of the inlet canal during a flood (133,950 cu. ft.); and (3) when the river returned to normal within its banks (234,900 cu. ft.). At no time were any *O. nosophora* recovered, even though a total of 466,050 cu. ft. of water was strained and examined.

[8] A check made in the spring of 1952 yielded 55 viable snails.

The third possibility was checked at the time of the fall pretreatment counts. Data accumulated from other areas of Japan, principally Kofu, indicated that eggs of *O. nosophora* laid in the spring and hatching even as late as June or July reach a length of 6.5 to 9 mm. with 7 or 8 whorls by the end of October or early November (Sugiura, 1933; 1950 Ann. Hist. Rept.). The 666 living and dead snails that were collected in the fall of 1951 conformed to this pattern. Consequently, it is concluded that the increase in snails that occurred between the spring post-treatment and the fall pretreatment counts was due largely to the reproduction of the surviving snails. Such a phenomenon points up the inherent difficulties which must be faced in any biological control project.

Cost of treating irrigation ditches. In the case of the snail vector of schistosomiasis in Japan, it should be noted that in most areas (except in river bottoms) the snails live along irrigation ditches much of the time above the water line. Apparently they do not seek the paddies as normal habitats but a few may be mechanically introduced by means of irrigation waters. Field observations suggest that most of these do not survive the winter. Consequently in considering

TABLE 2

Comparative costs of molluscacides per 1000 feet of ditch in Japan

CHEMICAL	COST
Sodium pentachlorophenate (Santobrite)	$1.30
DN-I	1.28 (McMullen *et al.* 1951a)
DN-111	3.50 (McMullen *et al.* 1951a)
Calcium cyanamide	2.50 (McMullen *et al.* 1951a)

treatment, the base line for calculating costs consists of the length of ditch involved. McMullen (1951a) calculated comparative costs of treating 1000 feet of ditches in Japan. These figures are listed in Table 2 and must be considered as only rough estimates.

According to these figures it will be seen that DN-I is slightly cheaper than the sodium pentachlorophenate (Santobrite). However, the ease of handling the new pellets, the greater specificity and apparently greater winter kill or residual effect, makes Santobrite the chemical of choice. Furthermore, it must be borne in mind that to date Santobrite is the only chemical which has received such extensive field trials, for at Nagatoishi over 12 miles of ditches, banks, and paddy margins were treated. For this reason it is recommended that the next step, which consists of a control project for the entire area endemic for schistosomiasis in Kyushu, utilize the same chemical, i.e., sodium pentachlorophenate (Santobrite).[9] Actually the total area is about the same size as the Kofu area as it embraces approximately 90 square miles, but both the incidence and density of the infection was higher in the Kyushu endemic area (1948 Ann. Hist. Rept.).

[9] Santobrite contains 79% sodium pentachlorophenate plus 11% sodium salts of other chlorophenols which is the equivalent of 90% sodium salts of chlorinated phenols as sodium pentachlorophenate. Dowicide G contains 4% less sodium pentachlorophenate and 2% more of the other soluble chlorophenols for a total of 88% active ingredients.

Proposed plan for controlling schistosomiasis in Kyushu. On the basis of a two-year demonstration control project of the intermediate snail host of *Schistosoma japonicum* in Kyushu, Japan, it is believed that the virtual eradication of schistosomiasis from Japan can be achieved at a reasonable cost with a 5–7 year program for each of the 5 endemic areas by treatment with sodium pentachlorophenate (Santobrite).

The necessity for such a control measure is emphasized by the following data pertaining to but one of the five endemic foci in Japan. Although only estimates, these data have been taken from figures furnished by officials of Fukuoka and Saga prefectures.

a. Estimated number of cases	28,617
b. Man-hours lost per year	24,213,120
c. Estimated cost of current treatment of infected persons per year	$177,938[10]
d. Wages lost per year	$2,522,200

Successful eradication of schistosomiasis would result in an eventual saving (by elimination of treatment and in wages saved) of a sum of almost $3,000,000 per year in this area alone.

Details of eradication plan for the Fukuoka-Saga area. Although it is unlikely that every single snail in the area would be destroyed, on the basis of our previous experience it is seen that definite, practical control would result from the following suggested plan, as applicable to this specific area:

(1) *Treatment.* It is recommended that treatment commence at the headwaters of infested streams and ditches upstream and that spraying (in accordance with a spraying formula) be systematically carried out. Treatment the following year should begin where the treatment of the previous year stopped. Each year the regions that were previously treated should be checked and resprayed where any surviving colonies of snails are found. In this way control would gradually approach the center (or downstream area) and over a period of 5–7 years eradication should be possible.

Sodium pentachlorophenate should be applied at least twice a year. In such cases a spring application is recommended prior to the extensive growth of vegetation. An additional fall treatment is desirable as this seems to have a marked residual effect on the survival of snails over the winter.

(2) *Labor.* Basic workers would be recruited in each village area. In addition, trained supervisors would be supplied by prefectural or city officials, as each area should be supervised by a qualified person.

(3) *Equipment.* Water cans may be utilized for part of the spraying. It is essential that pressure sprayers also be used for reaching under culverts, cutback banks, and into rodent burrows, etc. It is believed that such spraying equipment might be furnished for the entire project and utilized year after year.

(4) *Supplementary procedures.* It is important to utilize other devices which will facilitate contact of the chemical with the snail. This involves careful cleaning of the ditches, removing fall vegetation from the area to be sprayed, and if practical, reshaping the dirt irrigation ditch walls.

[10] Based upon the Japanese yen valued at the current rate of exchange Y360 = $1.00.

(5) *Cost of project.* Although it is possible that other chemicals would cost about as much, and might be equally effective, sodium pentachlorophenate (Santobrite) is recommended because of our experience with it on a large scale project at Nagatoishi. It is recommended that the Fukuoka-Saga area be treated twice a year. Enough additional chemical should be purchased to allow for re-treatment in the areas where colonies still persist in the second or even third year.

The figures quoted below are based upon the cost of treating the Nagatoishi area where a total of 12 miles or 63,360 linear feet of ditch were sprayed. Although the depth and width of the ditches varied it was estimated that the average dis-

TABLE 3

Estimated cost of sodium pentachlorophenate (Santobrite) required to control the snail host of Schistosoma japonicum *in the Saga-Fukuoka endemic area*

Ditches to be treated	23,265,648 sq. ft. or 534.1 acres.
Using 30 lbs./acre will need—16,023 lbs./treatment @ $0.25/lb.	$4,005.75
Treating twice a year will need—32,046 lbs.	8,011.50
For retreatment and treatment of unmapped ditches—10,000 lbs.	2,500.00
Total amount needed for 1st year—42,000 lbs.	10,500.00
Plus freight and shipping charges	—
Estimated total cost by years	
Estimated cost for 1st year (plus transportation)*	$10,500.00
Estimated cost for 2nd year	10,500.00
Estimated cost for 3rd year	10,500.00
Estimated cost for 4th year	5,250.00
Estimated cost for 5th year	5,250.00
Estimated cost for 6th year	3,500.00
Estimated cost for 7th year	3,500.00
Grand total (plus transportation costs)	$52,000.00

* Transportation costs are not included because these may vary over the seven year period.

tance from paddy edge, down and across the ditch, to the paddy on the opposite side was about 10 feet. Hence there were 633,600 sq. ft. or 14.5 acres that were treated. Using between 25–30 lbs. to the acre this would cost between $6.25 and $7.50 per acre to treat once. On the basis of figures supplied by Saga and Fukuoka prefectural officials it was estimated that there were 23,265,648 sq. ft. or 534 acres of ditch to be treated in this endemic area for schistosomiasis. Calculating 30 lbs. of sodium pentachlorophenate (Santobrite) per acre the cost would be approximately $4,000, at the current price of $0.25 per pound in lots of 30,000 lbs. or over. To this must be added freight and shipping charges. Table 3 summarizes these estimates by year for the entire project. It will be noted that the costs diminish as the project continues. This is because it is anticipated that after treating the entire area for three years only scattered colonies will remain. These must be sought out and destroyed.

It is possible to reduce the initial costs if it is planned to treat only one-third of the ditches of the total endemic area the first year. The following year such estimates would have to be doubled as the initial area would have to be retreated and another third of the total area sprayed for the first time. If this plan were followed the costs would reach their peak the third year when the entire area was under treatment.

This estimate covers only the cost of the chemical; it is assumed that other expenses such as equipment, supervisory personnel, etc., would be borne by the Japanese government. The overall coordinator might well be drawn from the Army or Public Health Service which have personnel familiar with this project.

While this project envisions the use of hand labor, it should be borne in mind that the practicability of spraying by helicoptor or small plane should be investigated. This would facilitate the control of snails in such areas as the Tone River valley.

SUMMARY

The feasibility of molluscacidal control of the amphibious snail, *O. nosophora*, by repeated applications of sodium pentachlorophenate (Santobrite) to 12 miles of ditch comprising the entire irrigation system of the 150 acre area of Nagatoishi has been demonstrated. This chemical was applied during both spring and fall of 1950 and 1951. The initial application reduced the snails to the extent of 98.1 per cent. The residual population apparently had a high reproduction potential as repopulation was considerable at the end of the first summer. The fall application in 1950 was apparently less effective than the initial spring kill; however, there appeared to be a residual effect which augmented the results secured in the fall.

In the spring of 1951 the pretreatment snail count was low and some repopulation occurred during the summer, but to a considerably less degree than during 1950. The fourth application gave an improved fall kill reducing the population by 99 per cent.

The final and definitive test of the feasibility of controlling this snail in Japan is outlined as the fourth step. This plan, including procedure of application and cost of chemical, is proposed for eradication of *O. nosophora* over the entire endemic area in Kyushu. Sodium pentachlorophenate (Santobrite) is proposed as the molluscacide of choice because of its relatively high efficiency and ease of handling and application.

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THREE NEW POTENTIAL INTERMEDIATE HOSTS OF HUMAN ONCHOCERCIASIS IN GUATEMALA[1]

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For years, *Simulium* (*S.*) *ochraceum*, *S.* (*S.*) *metallicum*, and *S.* (*L.*) *callidum* (Diptera, Simuliidae) have been considered by many authors to be the intermediate hosts of *Onchocerca volvulus* in Guatemala and Mexico. This concept has been based primarily on the presence of the three species in the disease zone and the finding of flies of these species naturally infected with filariid larvae. *S. ochraceum* has been thought to be the most important of the three in the transmission of human onchocerciasis, because of its predominantly anthropophilic tendencies. In an attempt to ascertain whether or not these three species are the transmitting agents in all parts of the onchocerciasis zones in Guatemala, a general survey was made, both within and outside of the known disease areas, to determine which species of Simuliidae exist and attack humans, thereby being possible intermediate hosts (Dalmat, 1951; Dalmat, in press).

During a three-year period, 3,992 collections of larvae and pupae were made from 510 streams throughout Guatemala. Over 160,000 larvae and more than 115,000 pupae were represented in these collections; of the latter, 78,500 were reared to adults. In addition, approximately 70,000 adult simuliids were collected while feeding on human subjects during 3,200 individual exposure periods. A study of these collections has indicated that, in some areas of the nearly inaccessible onchocerciasis zone of Huehuetenango (contiguous with the disease zone in Chiapas, Mexico), *S. ochraceum* is completely absent and apparently is replaced by *S.* (*L.*) *veracruzanum* as the principal anthropophilic species. In the lower altitude range of the Yepocapa onchocerciasis zone (below 2,500 feet), *S.* (*N.*) *exiguum* is the dominant human biter while in other regions of the zone *S.* (*L.*) *haematopotum* seems to replace *S. ochraceum* in importance. These findings stimulated a study of the potentiality of *S. veracruzanum*, *S. exiguum* and *S. haematopotum* as intermediate hosts of *Onchocerca volvulus*.

METHODS

Four volunteer subjects heavily infected with *Onchocerca volvulus* were chosen for the experiment, selection being based on the presence of numerous living microfilariae in skin biopsies. As a further proof of the suitability of these subjects, a test was made of their ability to establish normal infections in *Simulium metallicum*, a species in which the developmental stages of *O. volvulus* had been

[1] Part of a study jointly supported by the Laboratory of Tropical Diseases of the National Institutes of Health, U. S. Public Health Service, and the Pan American Sanitary Bureau, in cooperation with the Direccion General de Sanidad Publica of the Republic of Guatemala. The project was aided by a research grant from the National Institutes of Health, Bethesda, Maryland.

studied in the course of extensive experimental infections (Gibson, 1950, 1951). One hundred specimens of *S. metallicum* were fed on each subject, then maintained in the laboratory by the technique of Wanson, Henrard, and Peel (1946) as modified by Gibson (1951), and dissected as soon as they died. Results of these dissections showed that each of the four volunteer subjects was capable of producing normal infections in the flies, as judged by the abundant production of "sausage forms" on the fourth and subsequent days of infection.

Actual testing of *S. veracruzanum*, *S. exiguum*, and *S. haematopotum* as potential intermediate hosts of *O. volvulus* was carried out in zones of Guatemala where human onchocerciasis is not known to be endemic. The work with *S. veracruzanum* was done at Nebáj, Department of Quiché; that with *S. exiguum*, near Pastores, Department of Sacatepéquez; and *S. haematopotum* was tested at Finca El Llano near Palín, Department of Escuintla. The latter area is on the edge of the endemic zone and infected persons are occasionally found there. Autochthonous cases have not been reported, however, and it is doubtful if transmission of the disease occurs in that region.

Several hundred specimens of each of the three simuliid species were fed on the infected subjects, then maintained under laboratory conditions and dissected as soon as they died. Wild-caught flies were used since laboratory-reared simuliids consistently refuse to feed. It therefore was considered necessary to determine the natural infection existing in the fly population at each test area. Accordingly, several hundred wild (unfed) flies of each species were captured and dissected concurrently with those fed on the infected subjects.

RESULTS

Table 1 presents the results of dissections of the unfed flies (natural infection) and of the flies fed on infected patients (experimental infection). Only in the case of *Simulium exiguum* was an appreciable portion of the fly population found naturally infected with nematode larvae of the filariid type, and even in this case the significant difference between the natural and experimental infection rates leaves no doubt as to the validity of the latter. Nevertheless, it seemed worthwhile to attempt to determine the source of the 2.6 per cent spontaneous filariid infection in this species. Therefore, skin biopsies were examined from as many as possible of the humans and domestic animals residing in the area where the flies were captured. Table 2 summarizes the results of these examinations. On this basis it seems reasonable to assume that the natural infection found in *S. exiguum* was of bovine origin and probably was due to *Onchocerca gutturosa* Neumann (=*lienalis* Stiles?), since it is known that Guatemalan cattle are heavily infected with that parasite (Caballero, 1945; Gibson, 1951).

In the experimental infections, *Simulium exiguum*, *S. veracruzanum*, and *S. haematopotum* all proved capable of supporting the development of *Onchocerca volvulus* larvae. Table 1 lists the maximum developmental stage recovered in each species. These results are directly comparable with those obtained in experimental infections using *S. ochraceum*, *S. metallicum*, and *S. callidum*

(Gibson, unpublished data). Failure to recover developmental stages more than 334 hours after infection reflects the extreme difficulty of maintaining simuliid flies alive in the laboratory for more than two weeks, whether infected or not.

TABLE 1

Results of dissections of unfed flies (natural infection) and of flies fed on infected subjects (experimental infection)

SPECIES	NATURAL INFECTION			EXPERIMENTAL INFECTION							
							Maximum development observed				
	No. exam.	No. inf.	Inf.	No. exam.	No. inf.	Inf.	Days	Hours	No. flies	No. larvae	Stage
			%			%					
S. exiguum	463	12	2.6	514	83	16.2	12	288	2	2	Advanced "sausage"
S. veracruzanum	415	0	0.0	368	106	28.8	4	96	1	2	Pre-infective*
							9	200	1	3	Pre-infective
							13	312	1	27	Advanced "sausage"
S. haematopotum	1,276	2	0.2	759	47	6.2	13	310	2	9	Pre-infective
							14	334	1	2	Pre-infective

* This probably was a spontaneous (natural) infection, since the preinfective stage is never seen in experimental infections as early as 96 hours after infection.

TABLE 2

Examinations of skin biopsies near Pastores, Department of Sacatepéquez

SOURCE OF BIOPSIES	NUMBER EXAMINED	NUMBER SHOWING MICROFILARIAE	PER CENT SHOWING MICROFILARIAE
Man	207	0	0.0
Cattle	75	12	16.0
Horses	12	0	0.0

CONCLUSIONS

The foregoing experimental studies indicate that *Simulium exiguum*, *S. veracruzanum*, and *S. haematopotum* are capable of supporting development of *Onchocerca volvulus* when given the opportunity to feed on persons infected with microfilariae of that parasite, and that they are thus potential intermediate hosts. It is possible that these species actually are effective in the transmission of human onchocerciasis in certain parts of the endemic zones of Guatemala, where they are important or dominant human biters. These three species must therefore be included in any consideration of the epidemiology or control of human onchocerciasis in Guatemala, along with *S. ochraceum*, *S. metallicum*, and *S. callidum*.

SUMMARY

Several hundred specimens each of *Simulium exiguum*, *S. veracruzanum*, and *S. haematopotum* were allowed to feed on volunteers heavily infected with microfilariae of *Onchocerca volvulus*, and were subsequently dissected after various intervals of time. All three species proved capable of supporting the development of the parasite and must therefore be considered potentially capable of transmitting human onchocerciasis in those parts of the endemic zone where they replace or supplement *S. ochraceum*, *S metallicum*, and *S. callidum*, the three species commonly accepted as intermediate hosts.

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FAILURE OF AUREOMYCIN IN THE TREATMENT OF SCHISTOSOMIASIS[1]

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Fourteen cases of schistosomiasis were treated with aureomycin at the Santa Ana Clinic, Santurce, Puerto Rico. All patients were from 17 to 43 years of age except for one boy aged 8. All but the boy were discharging *Schistosoma mansoni* in the feces and the diagnosis was confirmed in 12 instances by rectal biopsy. The 8 year old boy was known to have had active schistosomiasis 2 years before and had been losing weight for several months along with a progressive enlargement of the abdomen. Physical examination revealed markedly enlarged liver and spleen. None of the patients were incapacitated at the time of treatment but 5 were underweight or anemic and one had a mild arteriosclerosis. All reported one or more of the following symptoms: epigastric pain and burning, weakness, dizziness, headache, diarrhea or slight difficulty of vision. Four of the patients had previously been treated with Fuadin and had subsequently relapsed.

TREATMENT SCHEDULE

Eleven of the patients received 2 grams of aureomycin daily for 14 days, 2 were given 1.5 grams daily for 21 days and the 8 year old boy was given 1 gram a day for 21 days. There were no toxic effects from the administration of the antibiotic in 9 instances but 3 patients had diarrhea and abdominal pain and 2 had some nausea.

RESULTS

The therapeutic response of the patients to aureomycin was disappointing. There was no evidence that symptoms were relieved or that the physical condition was improved. At the conclusion of the course of treatment, 7 of the patients still had ova in the stools. It was possible to observe for 2 to 3 months 5 patients whose stools had become negative after the aureomycin was discontinued. In 3 of these the feces had again become positive for *S. mansoni*.

CONCLUSION

Aureomycin did not prove to be an effective therapeutic agent in this group of patients with schistosomiasis.

[1] Acknowledgement is made of the cooperation of the Bayamon District Medical and Pediatrics Service and the assistance of Captain William Jahnes and Dr. Ross L. Gauld.

[2] Captain, M.C.

MODIFIED RESPONSE OF *ANOPHELES ALBIMANUS* TO DDT RESIDUAL HOUSE SPRAYING IN PANAMA

HAROLD TRAPIDO

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The Gorgas Memorial Laboratory, in cooperation with the former Tropical Disease Control Branch of the Office of the Surgeon General (U. S. Army) and the former Office of Scientific Research and Development, initiated experiments with DDT as a residual house treatment for the control of malaria in 1944. The area chosen for these experiments was a group of native villages on the middle Chagres River in Panama, where *Anopheles albimanus* is the malaria vector.

A description of the area, the climate and meteorology, the composition and habits of the mosquito population, and the results of the first fifteen months observations have been previously reported (Trapido, 1946). It was shown that following DDT residual spraying of dwellings the mosquitoes were affected in several ways. Within the houses three important effects were observed. First, there was a large reduction in the numbers of mosquitoes (of the order of 70 to as much as 99 per cent). Second, of those mosquitoes taken in treated houses there was a marked reduction in the percentage engorged with blood. Third, among such engorged mosquitoes as were taken in treated houses there was a very high 24 hour mortality rate. (Unengorged mosquitoes taken in treated houses survived for 24 hours in the same proportion as unengorged individuals from houses in untreated villages, thus demonstrating a selective killing of the mosquitoes potentially capable of transmitting malaria—the engorged individuals.) Apart from these striking effects noted in houses of a treated village there was also reported a "peripheral effect." That is, for a period of two or three weeks after treatment there was a large drop in the numbers of mosquitoes taken in a horse-baited stable trap in the center of the village, and even some transitory effect on a similar trap 300 feet from the village. There was no apparent effect in a trap 900 feet from the village.

A most complete malaria history of the villages in this area has been available for the twelve years before the introduction of DDT and the eight years since. For ten years thick blood films had been taken on all residents each month, and for the subsequent years thick film records on a bimonthly basis are available. Following the introduction of DDT residual spraying, there was a dramatic drop in the malaria incidence to a point where only an occasional positive was found. These positives in virtually all instances could be attributed to infections acquired away from the experimental villages, since these positive individuals were in almost all cases found to have been absent from the villages during several previous surveys. The history of the decline of malaria in these villages has been recorded by Dr. Herbert C. Clark in the annual reports of the Gorgas Memorial Laboratory (1945–51), and will not be considered further here.

With the decline of malaria in these villages the detailed semi-weekly house

captures of mosquitoes were discontinued at the end of 1946, although the routine DDT spraying was continued at approximately four month intervals. Despite the continued absence of any but occasional malaria positives the appearance of resistance to DDT and other chlorinated hydrocarbon insecticides in flies and certain culicine mosquitoes made it seem advisable once again to pick up the field observations to establish whether there had come about any change in the way *A. albimanus* in these villages responded to DDT residual house spraying after eight years of treatment of this sort. The present paper reports on the first four months of these renewed observations, January through April 1952.

FIELD OBSERVATIONS

General

A. albimanus is a species which enters dwellings at dusk and disappears from them at dawn. Just how much of the night is spent in houses has not been established. The diurnal resting places are out-of-doors. While it would be desirable to study the resting habits of these mosquitoes by means of window traps as has been done with certain anophelines in Africa, Iran and Southeast Asia, the house construction in Panama is such that this is not possible. As the writer has noted earlier (Trapido, 1946, p. 393) 90 per cent or more of the mosquitoes enter and leave the house in other ways than through the doors and windows. The houses of the experimental area are for the most part constructed with cane walls and palm thatch roofs. The bulk of the mosquitoes enter and leave either through spaces between the canes of the walls, or through the space between the tops of the walls and the overhanging eaves. The technique used for measuring mosquito abundance in the houses was therefore that described earlier (Trapido, 1946): a resident of each house in which collections were made was provided with a suction tube and a cage in which mosquitoes could be held alive. Collections were made for two and a half hours after dusk in the evening, and an hour before dawn in the morning. Mosquitoes collected by this means were held for twenty-four hours, when the dead individuals were removed from the holding cages, and the living ones subsequently chloroformed. Collections were made semi-weekly, on Monday and Thursday nights of each week. The results are thus comparable with those obtained during the 1944–1946 period.

While the area in which this experiment has been carried out is a tropical one in which mosquito breeding continues throughout the year, there are, nevertheless, fluctuations during the year in the abundance of the various species of mosquitoes, depending on variations in rainfall, humidity, the condition of the vegetation in the lacustrine situation in which the larvae develop, and other factors. These variations in climatic factors vary in some degree from year to year, as well as within a particular year. The absolute number of mosquitoes from year to year reflects these differences, but, in general, the abundance curves for different years are similar. The comparison of the mosquito populations of the present four month period of study have therefore been made with the same four month period for which data are available from the original experiment.

The routine of spray treatment maintained at the experimental villages over the period of years was the same as that employed when the experiment was initiated in 1944. The material used was five per cent technical grade DDT in kerosene. In the case of cane-walled houses the outside of the walls as well as the inside was sprayed since many mosquitoes alight on the outer walls and enter through crevices between the canes. The overhanging eaves of all houses were sprayed whether the roof was of palm thatch or galvanized iron since many mosquitoes also alight there on entering and leaving the houses. Inside the houses, all walls and furnishing were sprayed as well as the undersides of the roofs.

The present studies were made at two of the villages used in the earlier work, Gatuncillo and Santa Rosa. During the experiment previously reported, Santa Rosa had been used as a control and was not sprayed until it was sacrificed as a control in September 1947. The spraying of these villages on the 17th and 18th of January 1952 represented the thirteenth treatment for Santa Rosa in 52 months, and the twentieth treatment for Gatuncillo in 86 months. There is probably no village anywhere in the tropics which has been sprayed with DDT with such frequency over so long a period of time as Gatuncillo. It would therefore appear that if any resistance or behavior change were to develop in a natural population of anophelines, Gatuncillo would be a most favorable place to look for it.

Numbers of A. albimanus in DDT treated dwellings

When Gatuncillo was first sprayed with DDT at the beginning of November 1944 the effect on the gross numbers of mosquitoes in houses was immediate and dramatic. During the first month after this spraying the reduction in numbers of anophelines was from 81 to 100 per cent. (Trapido, 1944). The overall reduction in anophelines in houses during the four month period after the first spraying (Nov. '44–Feb. '45) was 88.3 per cent, after the second spraying (Mar.–June '45), 90.9 per cent, and after the third spraying (July–Oct. 1945), 96.8 per cent.

In 1952 control villages for comparison were no longer available, but we are able to make direct comparisons of the numbers of *A. albimanus* caught in houses of Gatuncillo itself during the first eighteen weeks (January to April) of 1945 and 1946, the first two years of treatment, with those taken during the same weeks of 1952. This comparison is made in Table 1. The average number of *albimanus* per night during the first eighteen weeks of 1945 was 4.6, that for 1946 was 1.4, and the average for the two years was 3.0. In striking contrast there were 21.7 *albimanus* per house per night during the comparable period of 1952, after twenty DDT treatments. (Data for the first three weeks of 1952 are lacking since the routine of house catching was not established until the fourth week of the year, immediately following the 20th spraying.) It is at once evident that there are many more *albimanus* entering treated dwellings than was the case during the first two years of the DDT spraying routine.

We may also examine with profit the data from Santa Rosa given in Table 2. This had been an unsprayed control village during the early work at Gatuncillo.

However the same routine of collecting data on the numbers of mosquitoes in houses was followed here for comparison with the then treated Gatuncillo. The figures given for the first eighteen weeks of 1945 and 1946 represent the normal prevalence of *albimanus* in houses of this village in the absence of DDT treatment. As mentioned above, this village was sacrificed as a control in September 1947, and on the 18th of January 1952 it experienced its thirteenth DDT treatment. The figures given for the second and third weeks of 1952 show the average numbers of *albimanus* per house per night for the two weeks before the thirteenth

TABLE 1

Average numbers of Anopheles albimanus *per house per night at Gatuncillo in the first and second years, and in the eighth year of DDT treatment*

(January through April of each year)

WEEK OF THE YEAR	1ST YEAR (1945)	2ND YEAR (1946)	AVERAGE OF 1ST AND 2ND YEARS	8TH YEAR (1952)
1st	5.8	3.5	4.7	—
2nd	4.0	2.8	3.4	—
3rd	3.3	1.3	2.3	—
4th	4.3	1.1	2.7	5.0
5th	4.3	2.0	3.2	6.0
6th	13.5	1.5	7.5	11.2
7th	4.0	2.0	3.0	14.2
8th	7.3	0.0	3.7	14.5
9th	7.3	4.0	5.7	18.2
10th	3.5	1.0	2.3	28.2
11th	2.3	2.0	2.2	22.8
12th	3.0	0.8	1.9	25.2
13th	1.3	0.6	1.0	21.7
14th	1.0	0.0	0.5	30.0
15th	6.8	0.0	3.4	55.7
16th	4.0	0.0	2.0	22.3
17th	4.5	0.3	2.4	32.2
18th	3.0	2.6	2.8	18.5
Average	4.6	1.4	3.0	21.7

treatment. It will be seen that they are essentially the same as the figures for the comparable weeks of 1945 and 1946 *when DDT had not been applied*. Of even greater significance are the figures for the subsequent weeks of 1952 immediately following the thirteenth spraying. It will be seen that the numbers of *albimanus* per house per night closely approximate the average numbers of this species in houses during chronologically comparable weeks of 1945 and 1946, prior to any treatment. The very close similarity in the numbers of *albimanus* in houses before the use of DDT and in the fifth year of its use may be summed up in the average figure for the first eighteen weeks of 1945 and 1946, 31.6, and the comparable figure for 1952, 34.6.

There can be no doubt from the data presented in these two tables that there

has been a very great change in the response of *A. albimanus* to DDT residual spraying of dwelling. These data appear plainly to demonstrate that DDT residual spraying no longer reduces the numbers of these mosquitoes in houses.

Engorgement of A. albimanus in DDT treated dwellings

While the killing of anophelines or otherwise reducing the numbers which enter dwellings is a desirable end in itself, this is only one of the ways by which the interruption of the transmission of malaria may be accomplished. The same

TABLE 2

Average numbers of Anopheles albimanus *per house per night at Santa Rosa, during two years prior to DDT treatment and in the fifth year of DDT treatment*

(January through April of each year)

WEEK OF THE YEAR	YEARS PRIOR TO TREATMENT			FIFTH YEAR OF TREATMENT (1952)
	1945	1946	Average of 2 years	
1st	20.8	10.3	15.6	—
2nd	28.2	30.5	29.4	20.2
3rd	36.0	17.5	26.8	41.2
4th	27.2	47.3	37.3	27.0
5th	58.3	43.7	51.0	67.5
6th	35.0	48.2	41.6	42.8
7th	47.0	36.0	41.5	58.2
8th	41.3	57.7	49.5	27.0
9th	18.5	53.0	35.8	38.2
10th	30.7	19.3	25.0	27.5
11th	42.7	29.7	36.2	35.8
12th	49.3	14.2	31.8	40.2
13th	34.8	10.7	22.8	36.2
14th	45.2	15.3	30.3	33.7
15th	50.5	3.8	27.2	27.8
16th	52.8	7.5	30.2	15.2
17th	29.5	5.3	17.4	19.3
18th	24.3	16.0	20.2	30.9
Average	37.3	25.9	31.6	34.6

end may be brought about by modifying the behavior of the anopheline so that it does not readily feed on humans. This effect on the behavior of mosquitoes entering DDT treated dwellings was observed following the first treatment at Gatuncillo in 1944 when the author wrote: "—of 135 mosquitoes collected in dwellings since the spraying of the village only four, or 2.9% have contained blood. It is indicated that mosquitoes entering the treated dwellings and contacting the DDT residue become so activated that they do not feed." (Trapido, 1944).

It is therefore of considerable importance to examine the present status of this phenomenon in order to determine whether DDT residual house treatment has any further value as a measure for the reduction of the malaria transmission

potential. In summarizing the results following the first three DDT treatments of Gatuncillo in 1944–45 it was shown that only 7.1 per cent of the *Anopheles* taken in treated dwellings were engorged compared with 47.4 per cent in untreated houses (Trapido, 1946, Table 4). During the period from February through April 1952, for which similar data are now available from Gatuncillo, we found 7.6 per cent of the *A. albimanus* to be engorged. It is therefore apparent that there has been no change in the effectiveness of DDT in reducing the proportion of anophelines which feed in houses at Gatuncillo.

We are also able to examine this effect of DDT at Santa Rosa. There we are able to compare the percentages of engorged *albimanus* from houses during the first four months of 1945 and 1946, before this village was sprayed, with the figures obtained during the period February through April 1952, following the thirteenth spraying of the village. These data are given in Table 3. We see that the summary figure of 4.5 per cent engorged for 1952 represents a considerable

TABLE 3

Per cent of Anopheles albimanus *engorged at Santa Rosa, during two years prior to DDT treatment and in the fifth year after DDT treatment*

	YEARS PRIOR TO TREATMENT		FIFTH YEAR OF TREATMENT (1952)
	1945	1946	
January	52.8	47.4	—
February	33.5	34.5	1.2
March	37.0	28.9	5.7
April	42.6	41.7	6.9
Average	42.1	39.2	4.5

drop from the 42.1 per cent in 1945 and 39.2 per cent in 1946. These results are quite similar to those obtained at Gatuncillo, and confirm the observation made there that DDT residual spraying over a period of years continues to reduce the proportion of *albimanus* which feed in dwellings just as it did initially.

Selective mortality of engorged A. albimanus in DDT treated dwellings

The third effect observed on the mosquitoes of treated dwellings at the Chagres River villages in the early work during 1944 to 1946 was the high mortality rate of engorged mosquitoes. During the four month periods following the first three DDT treatments of Gatuncillo in 1945 the average survival of engorged anophelines was only 4.2 per cent, while 38.3 per cent of the engorged individuals from the then unsprayed villages of Santa Rosa and Guayabalito survived for a similar period. On the other hand, there was no material difference in the 24 hour survival rates of unengorged anophelines from houses in treated and untreated villages at that time. At Santa Rosa and Guayabalito, which were then untreated, the 24 hour survival rate for unengorged anophelines was 36.7 per cent, while at Gatuncillo, which was treated, the survival rate was 41.7 per cent (Trapido,

1946, Tables 5 & 6). In 1952, at Gatuncillo, there was no longer a marked difference in the 24 hour survival rate of engorged and unengorged *Anopheles albimanus*, the rate for engorged individuals being 35.2 per cent and for unengorged 30.7 per cent.

The writer's interpretation of the high mortality among engorged mosquitoes during the early work at the Chagres River villages was that since they carried the weight of a blood meal, the engorged mosquitoes tended to rest longer and were thus more likely to accumulate a fatal dose of DDT. This selective killing of engorged anophelines was considered an important factor in lowering the malaria transmission potential. The survival rates derived from the data obtained during the first four months of 1952 would indicate that for reasons which are not superficially apparent the selective killing of engorged *albimanus* in DDT treated houses at Gatuncillo no longer occurs.

DISCUSSION

It was unfortunately not possible to re-establish horse-baited stable traps at the experimental villages until some weeks after the January 1952 spraying had been completed. Thus it is not possible at this time to report whether or not the transitory reduction of mosquitoes in treated village areas, an effect observed in 1944 and 1945, is still apparent. The routine semiweekly catches of mosquitoes in stable traps at Santa Rosa were begun in the ninth week, and at Gatuncillo in the twelfth week of 1952. The catches from these traps are sufficient to indicate that while the general level of anopheline abundance was somewhat higher than that recorded for the same time of year in the 1945 to 1947 period, the increase was not sufficient to account for the relatively large numbers of anophelines in houses of treated villages.

There appear to be two possible explanations of what may have happened to modify the response of *A. albimanus* to DDT treated houses at the Chagres River villages. The one is that these mosquitoes may have become resistant; the other is that through a selective killing off of those least irritated by DDT, the population now largely consists of individuals with an enhanced predisposition to irritability on minimal contact with DDT, which results in their having only fleeting sublethal contacts with DDT treated surfaces. It may also be that both these factors are involved, the one complementing the other. In this view, the present population would have developed both hyperirritability and some resistance: the hyperirritability would ensure that only minimal contacts were had with DDT treated surfaces, while the resistance, however slight, would raise the threshold of the time of contact for the accumulation of a lethal dose of DDT to a point where more individuals survived.

One or another or both of these factors operating in conjunction would explain the fact that the numbers of *albimanus* in houses are no longer reduced as they were in the first years of the use of DDT. There is no way to measure resistance on the basis of the field observations considered in the present paper, but certain of the observations strongly support the interpretation that an enhanced irritability to DDT has appeared. In the 1944 to 1946 period a preponderance

of the *albimanus* taken in houses would be found on walls, furniture, and on cross beams throughout the interior of the houses. In the course of making the night collections during the present study in 1952, it was observed that while there were many mosquitoes in the treated houses they were largely restlessly moving about in the air. The native collectors are adept with suction collection tubes and are able to collect these mosquitoes out of the air. Yet another behavior difference noted on the part of *albimanus* in treated houses in the present study has been their greater positive phototropism. In the native houses at the Chagres River villages the usual source of light at night is either a conventional kerosene lamp or an open flare-like lamp consisting of a wick made of a corn cob or similar absorbent material stuck in a bottle of kerosene. There is always a considerable accumulation of mosquitoes hovering about this light, and even flying through it, so that many mosquitoes are singed and found dead or moribund on the table around the foot of the lamp. Laboratory studies to measure quantitatively a possible resistance and enhanced irritability and response to light of *albimanus* from these treated villages, against a colony which has had no contact with DDT, are now in progress and will be reported in a separate paper.

The second effect of DDT, that of reducing the feeding of *albimanus* has apparently not been modified over the period of years, and seems to be at present the principal factor in reducing possible malaria transmission in DDT treated dwellings.

The ultimate success of a method such as that of DDT residual spraying of dwellings is measured by the malaria rates. As yet we have no evidence that malaria is returning to these villages. However, with the malaria seed bed reduced virtually to nil, it is to be expected that there would be some lag in time before fresh infections contracted elsewhere were introduced to infect the mosquitoes and again start the chain of transmission. A variable other than the mosquito numbers and behavior which might have affected the malaria rates was introduced at the experimental villages in 1946 when the drug routinely administered to persons found positive for malaria on the bimonthly blood thick film surveys was changed from atabrine to chloroquine.

SUMMARY

From observations conducted in 1944 to 1946 in a group of native villages on the middle Chagres River in Panama it had been concluded that the DDT residual spraying of dwellings initially reduced the malaria transmission by *Anopheles albimanus* in houses in three ways. During the first four months of 1952, following the twentieth spraying of one of these villages and the thirteenth spraying of another, it was found that two of these effects are no longer apparent. The gross numbers of *A. albimanus* in dwellings are no longer drastically reduced as was formerly the case, and the selective killing of engorged *albimanus* is no longer evident. A large reduction in the percentage of *albimanus* which successfully engorge with blood in DDT treated houses is still present to about the same degree as at the time of the initial observations in 1944 to 1946. This last appears to be the principal way in which *albimanus* is affected by DDT to modify the malaria transmission potential at the present time.

A possible explanation is given of how this change in the way *albimanus* responds to DDT residues may have resulted from the selection of a population which has become resistant and/or hyperirritable to DDT. Laboratory experiments to measure the contribution of each of these factors quantitatively are in progress.

The modification in the response of *A. albimanus* to DDT residual house spraying has not as yet been reflected in any rise in malaria rates at these villages.

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CHRONIC ORAL TOXICITY AND RELATED STUDIES ON ANIMALS WITH THE INSECTICIDE AND PYRETHRUM SYNERGIST, PIPERONYL BUTOXIDE

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INTRODUCTION

Piperonyl butoxide is a synthetic organic insecticidal material, first prepared by Wachs (1947), which has gained widespread use since its introduction into practical insecticide formulations. It has been used primarily in synergistic combinations with pyrethrins (Dove 1947). Abundant evidence has shown that there is a striking increase of the insecticidal properties of pyrethrins when these expensive materials are combined properly with the relatively inexpensive Piperonyl butoxide. This marked synergism has permitted the economic use of pyrethrins in a much wider range of applications than was possible previously.

Earlier studies have uniformly indicated the relative nontoxicity of Piperonyl butoxide to warm-blooded animals. Lehman (1948, 1949) listed Piperonyl butoxide as the least toxic of some 20 or more insecticidal materials which had been studied by the Division of Pharmacology, Food and Drug Administration. The relatively low acute toxicity of Piperonyl butoxide to various species was also demonstrated by the studies of Sarles *et al.* (1949).

In order critically to evaluate the toxicology of Piperonyl butoxide, acute, subacute, and two-year chronic experiments in rats, and chronic experiments in dogs were begun in 1947. One experiment dealt with the toxicity of a combination of Piperonyl butoxide and pyrethrins. The translation of the results into terms of projected toxicity in humans was the ultimate objective of the present study.

MATERIALS AND METHODS

The test material was undiluted, commercial "Technical Piperonyl Butoxide." This product contains not less than 80 per cent of 3,4-methylenedioxy-6-propylbenzyl butyl diethylene glycol ether and 20 per cent of compounds which are closely related in structure and properties. An alternative name of (butyl carbityl) (6-propyl piperonyl) ether has received official acceptance by the Production and Marketing Administration, U. S. Department of Agriculture, for use in labeling.

Technical Piperonyl butoxide is a pale amber, clear liquid with a mild characteristic odor and slightly bitter taste. The specific gravity is between 1.04 and 1.07 at 20°C./20°C.; boiling point is approximately 180°C. at 1 mm. It is insoluble in water but readily soluble in alcohol, benzol, petroleum oil and most organic solvents as well as in liquified gases of the Freon type.

The pyrethrins are the active insecticidal components of pyrethrum flowers

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and were used in this study in the form of a 20 per cent commercial extract. In one group of tests a ratio of 1 part pyrethrins to 6 parts Piperonyl butoxide by weight was used, representing an approximate average of the several ratios of these two materials used in commercial applications.

Dosages of Piperonyl butoxide and of the Piperonyl butoxide-pyrethrins combination were measured by volume. Because the specific gravity is close to unity, the concentrations of chemicals are frequently referred to in this paper in terms of per cent and parts per million, without correction for the gravity. In the chronic rat experiment the materials were diluted with 50 ml. of acetone before being added to 1000 gm. portions of Purina Laboratory Chow Meal. After thorough mixing with an electric food mixer the treated food was spread thinly to permit evaporation of the acetone. The food of the control group was treated similarly with acetone alone. Adequate amounts of food and water were available continually. Dogs were fed untreated Purina Laboratory Chow Checkers twice daily in total daily amounts of one-half pound for puppies, three-fourths pound for small dogs and one pound for large dogs; dosage of chemical was by capsule.

Stock albino rats were from a reputed Wistar strain; general health and reproduction were good. The animals were kept in large rooms in which the effects of outdoor temperature and humidity were free to operate during the summer months. Dogs were raised from mongrel litters. All animals were caged separately except the pairs of rats in the chronic experiment; wood shavings were placed in the cages of paired rats.

Cell counts were made on blood from tail veins of rats and ear veins of dogs.

Weights of organs of rats were determined either before or after fixation whereas weights of organs of dogs were determined before fixation. Tissue blocks and organs were fixed in 10 per cent formalin and impregnated in paraffin. Sections were stained routinely with Harris hematoxylin and 70 per cent alcoholic eosin-phloxine.

Microscopic examination of tissues of all animals in the critical experiments and of the animals dying in the other experiments included pieces of all the viscera and endocrine glands, brain and spinal cord, vertebral marrow, striated muscle and other indicated tissues. In sacrificed animals the important organs and indicated abnormal tissues were studied. Wright stains were made of smears of exudate from the ears, sinuses and elsewhere as indicated. No bacterial cultures were made.

EXPERIMENTS

In each experiment the Piperonyl butoxide was administered by way of the alimentary tract: by stomach-tube, fed in food, or by gelatin capsule. The chronic rat experiment was the main experiment; it was divided into six groups. The acute and subacute rat experiments were supplementary whereas the chronic dog experiment and the test on goats served to corroborate the findings of the rat experiment in larger, non rodent species of experimental, warm-blooded animals. The chronic dog experiment paralleled the rat experiment on a smaller scale.

A preliminary chronic toxicity rat experiment indicated the dosages which would be non-toxic, slightly toxic, moderately toxic, and fatally toxic, and, in a general way, served as a pilot test. Elaboration of this preliminary, two-year experiment is omitted because the limited results were duplicated entirely by the final experiment.

Reproductive efficiency of rats was determined by continuously breeding pairs and replacing dead mates by rearrangement of survivors, and by noting the number, size, and condition of the litters. The male was removed during the four week period of nursing. In order to test for any possible accumulative toxic effect of Piperonyl butoxide on the growth and reproduction of the second rat generation and on the growth during weaning in the third generation, the first litters of the last two females of the first five groups were saved (92 rats) and paired at weaning to produce the third generation of first litters (266 rats). The rats of the second and third generations were maintained on the test diets of the mothers. Effect of the chemical on the reproduction of dogs was tested only in one heavily dosed animal.

The animals were observed daily in regard to general condition. Each week, food consumption of rats was determined by deducting the estimated amount of food wasted from the total amount used, and the per cent of the control value was calculated. Body weight was determined weekly. Blood cell counts were made at intervals of approximately two months on the last two males of each rat group and on all dogs; differential leucocyte counts were discontinued after the early months when they were found to yield consistently normal values.

Autopsies were performed on all animals, including those of the second and third generations of rats. One male and one female rat were sacrificed at 26 and 52 weeks from each of the first five groups of the chronic experiment for comparative tissue studies. These and the surviving animals at the termination of the experiments were etherized and killed by exsanguination. None of the other animals in the chronic rat and dog experiments was sacrificed during the experiments before the event of natural or toxic death. Microscopic study of the livers of rats of the second and third generations respectively was limited to those with the four largest livers in groups IV and V, and weanlings of the second generation of group V.

1. *Acute toxicity rat experiment.*—Agent: Piperonyl butoxide. Duration: 2 days. Number of animals: 11; number treated: 9 (5 females and 4 males); 2 controls (1 male and 1 female). Average starting weight: males 264 gm.; females 170 gm. Administration: by stomach-tube. Dosage: the single dose given, following an overnight fast, ranged from 12.5 ml./kg. to 37.5 ml./kg. Even with the great dosages of 12.5 ml./kg. and 25 ml./kg. no death occurred under 24 hours. Therefore, two rats were fed 37.5 ml./kg. and sacrificed at seven and one-half hours. Deaths during the second 24 hours occurred at irregularly spaced intervals.

2. *Subacute toxicity rat experiment.*—Agent: Piperonyl butoxide. Duration: 31 days. Number of animals: 14; treated: 12 (6 males and 6 females); 2 controls (1 female and 1 male). Average starting weight: males 207 gm.; females 155 gm. Administration: by stomach-tube. Dosage: single daily doses six days a week.

The first seven doses were at 2.5 ml./kg. Two animals died on the third and fourth days. Owing to improvement of others after initial toxicity, the second seven doses were at 3.5 ml./kg. Little toxicity was noted so the remaining doses were at 5ml./kg. Deaths were spaced from the 17th to 24th days; the next and last death was at 31 days.

3. *Chronic toxicity rat experiment.*—Agent: Piperonyl butoxide mixed with food; in Group IV Piperonyl butoxide and pyrethrins were mixed with food. Duration: 2 years. Number of animals: 24 rats in each of six groups; males and females equally divided and opposite sexes paired throughout experiment. Average weight at start of experiment: males 44 gm.; females 44 gm. Five dosage groups: (see Table 1). Rats in Group VI were started on the Group III ration for one week in an attempt to adapt the rats to the treated food. The ratio of pyrethrins to Piperonyl butoxide in Group IV was 1:6.

4. *Chronic toxicity experiment on dogs.*—Agent: Piperonyl butoxide by capsule. Duration: 1 year. Number of animals: 20. Treated: 16 (9 males and 7 females); 4 controls (2 males and 2 females). Age at start of experiment: for 19 dogs, varied between 15 and 40 weeks; age of other dog was estimated at one year. Weight at start of experiment: Varied greatly. Four dosage rates: (see Table 7). Except for the smallest doses the total daily dosage was divided into an early morning and late afternoon capsule six days a week. The actual total daily dose varied with changes in body weight, except for the first dog in each group which received a daily dose of constant size.

5. *Chronic toxicity experiment on goats.*—A mature goat (see Table 7) was fed a constant daily dose of 2.0 ml. of Piperonyl butoxide by capsule six days a week for a period of one year (a dosage rate equivalent to 0.1 per cent of Piperonyl butoxide in the food). The dosing was started four days after the dam had given birth to a female kid, and both goats were observed for a period of one year for any signs of direct toxic effects upon the dam or of indirect effects upon the nursing kid through possible presence of Piperonyl butoxide in the dam's milk.

6. *Biochemical balance tests.*—In the first biochemical balance test undiluted Piperonyl butoxide was fed orally by capsule in a single dose of 8.45 gm. to a dog weighing 16.15 kg. (equivalent to about 500 mg./kg.). Feces and urine were collected for four days. The second test was a repetition of the first test on the same dog and followed an interval of six weeks. At this time the dog weighed 15.42 kg. and received 7.71 gm. of Piperonyl butoxide. Feces and urine were collected for seven days.

Samples of feces were dried at reduced pressure, then ground and extracted overnight with hexane. After removal of hexane by distillation, the residue was made to a definite volume with deodorized base oil. Samples of urine were thoroughly extracted with hexane in separatory funnels after which the hexane fraction was treated as above. Content of Piperonyl butoxide was quantitatively determined by the formation of a characteristic blue color with a mixture of tannic, phosphoric, and acetic acids, which color was read in a colorimeter using a filter of 625–635 millimicron wave length range (Jones *et al.* 1952).

RESULTS

I. Rat experiments

Symptoms: In the two-year chronic toxicity experiment the rats fed rations containing 0.01 per cent and 0.1 per cent of Piperonyl butoxide, and 0.1 per cent of Piperonyl butoxide plus one-sixth as much pyrethrins, did not differ significantly from the control rats in appearance or activity, and showed no symptoms of chronic toxicity. The rats of the 1.0 per cent group in this experiment con-

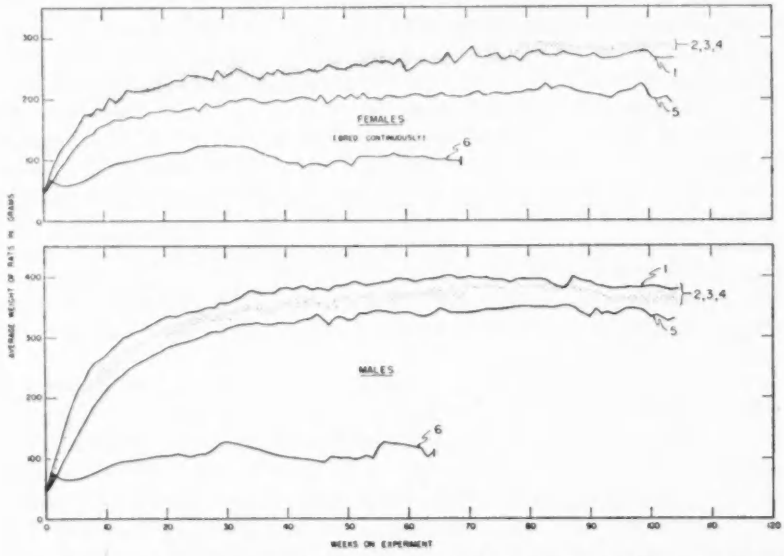

FIG. 1. Average weight curves for groups of 12 rats in two-year experiment on chronic toxicity of Piperonyl Butoxide. Concentration of Piperonyl Butoxide in ration was 1—none; 2—0.01%; 3—0.1%; 4—0.1% (plus 0.0167% pyrethrins); 5—1.0%, and 6—2.5%.

The curves for groups 2, 3, and 4 are represented by the stippled area because they did not differ significantly from one another and were too intertwined to be indicated separately at this scale.

stantly wasted food and were always slightly smaller than the control rats. Anorexia was outstanding in the rats of the chronic experiment which were fed the highest concentration (2.5 per cent) of Piperonyl butoxide in their food and resulted in severe stunting and cachexia.

In the acute toxicity experiment the symptoms resulting from a single very large dose of Piperonyl butoxide included anorexia, unsteadiness, rough coat, watering of eyes, irritability, prostration, coma, and death.

In the repeatedly dosed rats of the subacute experiment symptoms were delayed and included terminal anorexia, loss of weight, prostration, coma, and death.

Change in body weight and food consumption (Figure 1 and Table 1): No significantly subnormal gain in weight, nor markedly subnormal food consumption, in comparison to the control group, occurred in the first three test groups (II–IV) of the chronic toxicity experiment. Decrease in growth in the 1.0 per cent group (V) was related to subnormal consumption of food (78 per cent of control amount). Stunting, and cachexia, in the 2.5 per cent group was related to extremely subnormal consumption of food (37 per cent of control amount). The initial loss of weight of the rats of the 2.5 per cent group was, no doubt, caused by the rapid onset of severe anorexia.

TABLE 1

Weight and food consumption data, two-year rat experiment on chronic toxicity of Piperonyl Butoxide

SEX	GROUP NO.	PIPERONYL BUTOXIDE IN FOOD		AV. INITIAL WT. (12 4-WEEK-OLD RATS)	FIRST 12 WEEKS		FIRST 52 WEEKS		AV. RELATIVE RATE OF FOOD INTAKE BY BOTH SEXES‡
					No. of rats	Av. gain in body wt.	No. of rats	Av. gain in body wt.	
		%	*p.p.m.*	*gm.*		*gm.*		*gm.*	%
M	I	0	0	50	11	240.1	10	338.2	100
M	II	.01	100	49	12	229.1	11	313.7	*93.4*†
M	III	.1	1000	46	11	221.5	10	327.6	102.0
M	IV	.1*	1000*	46	12	*204.2*†	10	314.9	*94.0*†
M	V	1.0	10,000	47	12	*183.6*†	11	*292.3*†	*78.0*†
M	VI	2.5	25,000	45	8	*21.1*†	2	*26.0*†	*37.0*†
F	I	0	0	46	12	149.3	10	210.7	
F	II	.01	100	46	12	154.3	10	210.2	
F	III	.1	1000	43	12	145.2	11	212.4	
F	IV	.1*	1000*	41	12	143.2	10	207.5	
F	V	1.0	10,000	44	12	*122.6*†	9	*155.8*†	
F	VI	2.5	25,000	43	8	*25.0*†	2	*25.5*†	

* Plus one-sixth as much pyrethrins.

† Italicized averages are only ones significantly different (showing a difference which is four or more times the probable error) from the controls.

‡ Values represent average of 104 weekly comparisons for Groups I–V, and of 68 weeks for Group VI.

Reproductive efficiency of rats of first generation (Table 2): No decrease in reproductive activity occurred in the first three groups (II–IV) fed treated food for two years as compared to the control group. A significant decrease in reproductive efficiency of the 1.0 per cent group was indicated by a decrease in per cent of litter-bearing females, a longer average period of time before the first litter was born, decrease in number of litters per female, decrease in average weight of weanlings, and poorer general condition of the litters. None of the females of the 2.5 per cent group reproduced.

Second and third generation test for accumulative toxicity (Table 3): With the exception of one female dying and one female accidentally killed in the second generation, all weaned rats assigned to the second and third generation test

developed to maturity, and nearly all second generation females gave birth to third generation litters in which a high per cent of young survived to weaning age. One female of the 1.0 per cent group gave birth to a litter of runts.

TABLE 2

Reproduction data, two-year rat experiment on chronic toxicity of Piperonyl Butoxide

GROUP NO.	PIPERONYL BUTOXIDE IN FOOD	TOTAL LITTERS BORN NO.	FEMALES BEARING LITTERS	AV. DAYS UNTIL FIRST LITTER	AV. NUMBER OF LITTERS BORN PER FEMALE	AV. DAYS BETWEEN LITTERS	AV. NUMBER OF YOUNG PER LITTER	AV. PER CENT OF LITTER RAISED TO 4 WEEKS AGE	AV. OF MEAN WT. OF YOUNG IN LITTER AT 4-WEEKS AGE
	%		%						gms.
I	0	90	91.7	59.4	9.0	58.2	8.1	77.8	42.2
II	.01	66	83.3	60.1	7.1	56.2	8.0	74.0	44.5
III	.1	98	91.7	60.2	9.9	56.7	8.4	79.0	40.5
IV	.1*	70	91.7	58.9	7.8	55.2	8.1	82.5	41.6
V	1.0	38	58.3	*83.4*†	*5.3*†	60.4	7.4	74.1	*23.7*†
VI	2.5	0	0	—	—	—	—	—	—

* Plus one-sixth as much pyrethrins.

† Italicized averages are only ones significantly different (showing a difference which is four or more times the probable error) from the controls.

TABLE 3

Summary of data on second and third generation, two-year rat experiment on chronic toxicity of Piperonyl Butoxide

GROUP NO.	PIPERONYL BUTOXIDE IN FOOD	SECOND GENERATION*						THIRD GENERATION		
		Number weaned and in test†	Males		Females			Av. number of young per litter	Av. per cent of litter raised to 4-wk. age	Av. wt. at 4-wk. age
			Weeks in test	Av. wt. when killed	Weeks in test	Av. wt. when killed	Average per cent bearing litters			
	%			gms.		gms.				gms.
I	0	15	14	262	19	212	84§	8.2	82	40
II	0.01	17	14	280	19	210	100	10.4	99	38
III	0.1	21	14	233	19	187	100	9.4	100	37
IV	0.1‡	14	20	311	22	210	62	5.5	46	36
V	1.0	14	22	218	24	166	88	5.0	79	28

* Values given are for two litters in all cases.

† All rats survived throughout the test, except in Group II in which one female died in the 18th week and one female with litter accidentally was killed in 14th week.

‡ Plus one-sixth as much pyrethrins.

§ One female mistakenly thought pregnant and removed from mate too soon.

Hematology: No abnormal blood and differential counts were obtained with the exception of several decreased red blood cell counts in one natural fatality in the 1.0 per cent group and in one rat in the 2.5 per cent group. There was no evidence of hemorrhagic tendencies.

Mortality: In the first five test groups of the chronic experiment all deaths

(see Table 4) resulted from natural diseases. The increase of the rate of natural death in the 1.0 per cent group indicated a predisposition to natural death by pre-existing toxicity of Piperonyl butoxide.

All animals in the 2.5 per cent group died primarily of liver damage due to Piperonyl butoxide. Deaths in the 2.5 per cent group occurred between the fourth and 68th weeks. The times of death fitted into three well defined categories suggesting that the variable individual susceptibility to the toxic effects of Piperonyl butoxide was in reality a phenomenon of groups: 1) eight animals died between the fourth and ninth weeks; 2) five animals died between the 20th and 26th weeks; 3) 11 animals died between the 41st and 68th weeks.

TABLE 4

Accumulative mortality data, two-year rat experiment on chronic toxicity of Piperonyl butoxide

GROUP NO.	PIPERONYL BUTOXIDE IN FOOD	SEX	MORTALITY AFTER—			
			26 weeks	52 weeks	78 weeks	104 weeks
	%		%	%	%	%
I	0	M	8.3	9.1	10	30
		F	0	9.1	10	20
II	.01	M	0	0	30	40
		F	0	0	0	30
III	.1	M	8.3	9.1	20	40
		F	0	0	10	50
IV	.1*	M	0	9.1	20	50
		F	0	9.1	20	20
V	1.0	M	0	0	10	60
		F	8.3	18.2	30	60
VI	2.5	M	66.7	83.3	100	100
		F	41.7	83.3	100	100

* Plus one-sixth as much pyrethrins.

In the control group three of five deaths occurred in the second year; 30 of the 35 deaths in the first four test groups also occurred in the second year (see Table 4). The causes of these 40 natural deaths were: upper respiratory tract infection, 5; lower respiratory tract infection, 5; combined respiratory tract infection, 2; upper respiratory tract infection with meningitis, 2; upper respiratory tract infection with cellulitis, 2; visceral abscesses and ulcers, 3; overgrown incisor teeth with osteomyelitis of facial bones, 2; severe hemorrhages, 2; atrophy of parenchymal cells of brain, 3; heat stroke, 4; sarcomas, 4; glioma, 1; huge benign connective tissue tumors, 2; adamantinoma of mandible, 1; adenoma of hypophysis with hemorrhagic rupture, 1. The unaccounted death was attributed to a functioning islet cell adenoma of the pancreas inasmuch as the tumor was 3 mm. in

diameter, appeared similar microscopically to functional adenomas of humans and the autopsy was without other findings. Presumptive evidence of bartonellosis occurred in 9 natural fatalities (6 in Group V) and in 4 of the induced deaths of the 2.5 per cent group.

With the exception of the sacrifice of the first two animals in the acute experiment and of two animals near death in the subacute experiment, all animals in these two experiments were permitted to die. Each death in these two experiments was attributed to liver damage by Piperonyl butoxide; in deaths during the first four days an additional cause of secondary importance was destruction and damage to ganglion cells of the brain stem.

Morbidity: No evidences of natural disease were found in the 20 rats sacrificed at 26 and 52 weeks from the first five groups of the chronic toxicity experiment (see Table 1) with the exception of findings suggestive of bartonellosis in each rat. In the three of these groups (III, IV, V), in which Piperonyl butoxide produced toxic lesions, the average number of rats per group suffering from natural disease at the termination of the experiment among the 44 sacrificed two-year rats was six (41%); the average number among the 38 surviving rats in the control group and group II was 11 (58%). Part of this difference in the morbidity rate among the survivors was due to accentuation of natural disease in the 1.0 per cent group and, hence, to the greater number of diseases becoming fatal during the course of the experiment.

One rat in the 2.5 per cent group developed simultaneously a hepatic cell adenoma and a hepatic cell carcinoma; the carcinoma metastasized to the abdominal lymph nodes, lungs and mediastinum; no other tumors occurred in this group. Five rats suffered severe concomitant natural disease. The diseases were 1) large cerebral abscess and associated purulent sinusitis, 2) large cerebellar abscess, 3) multiple abscesses of the liver, 4) multiple bronchial abscesses and pneumonia and 5) subacute ulceration of the cecum. Small bronchiectatic abscesses were found in two rats.

The incidence of the natural diseases in the 60 (29M, 31F) sacrificed two-year rats were: upper respiratory tract infections, 16; lower respiratory tract infections, 7; visceral abscesses and ulcers, 6; chronic nephritis, 5; benign breast tumors, 12; benign endocrine gland tumor, 13; benign connective tissue tumors, 6; liposarcoma, 1; hepatoma (in control and 1.0 per cent group), 2. Multiple occurrences account for the excessive incidence of disease. Presumptive evidence of bartonellosis occurred in all sacrificed animals.

The incidence of benign breast and endocrine gland tumors in 93 rats of which 33 (19M, 14F) died in the second year and 60 (29M, 31F) were sacrificed at the end of two years was: breast, 14; hypophysis, 8 (6M, 2F); adrenal, 9 (2M, 7F); and islet of Langerhans adenoma, 1. Considering only the 60 sacrificed two-year rats the incidence was: breast, 12; hypophysis, 5; and adrenal, 8.

Organ weights: The data on organ weights, summarized in Table 5, are given in terms of the per cent of the body weight represented by each organ, and are based on all rats in each group, including the fatalities and pairs of rats sacrificed at 26 and 52 weeks as well as the two-year survivors, except those animals in which large growths or abscesses grossly affected the body and organ weights.

The average per cent weight of the various organs deviated significantly from the control values only in the case of the livers and kidneys of the two groups of rats fed the highest concentrations of Piperonyl butoxide (1.0 and 2.5 per cent). In the case of the 2.5 per cent group of first generation rats, the livers and kid-

TABLE 5

Data on organ weights, two-year rat experiment on chronic toxicity of Piperonyl Butoxide

GROUP NO.	PIPERONYL BUTOXIDE IN FOOD	GENERATION†	AGE (WEEKS)		PER CENT OF BODY-WEIGHT REPRESENTED BY—							
					Liver		Kidneys		Spleen		Testes	
			Range	Av.	No. rats	Av.	No. rats	Av.	No. rats	Av.	No. rats	Av.
	%					%		%		%		%
I	0	First	7–104	85	20	3.9	20	1.0	20	0.21	9	1.2
II	0.01	First	26–104	85	19	3.5	19	1.0	19	0.21	10	1.3
III	0.1	First	9–104	83	23	4.1	22	1.1	23	0.25	10	1.2
IV	0.1*	First	26–104	85	23	4.0	23	1.0	23	0.22	11	1.2
V	1.0	First	25–104	80	21	*5.8*‡	21	*1.4*‡	21	0.26	12	1.3
VI	2.5	First	4– 68	31	19	*15.0*‡	10	*2.3*‡	8	0.19	3	0.6
I	0	Second	14– 20	17	4	4.3	3	0.9	4	0.16	2	1.3
II	0.01	Second	14– 19	16	6	3.9	6	1.1	6	0.18	3	1.5
III	0.1	Second	13– 20	17	4	4.8	2	0.9	4	0.25	2	1.7
IV	0.1*	Second	13– 23	19	6	4.6	7	1.0	7	0.16	4	1.4
V	1.0	Second	14– 27	23	8	*6.1*‡	4	0.9	5	0.18	3	1.6
I	0	Third	—	4	6	5.0						
II	0.01	Third	—	4	8	4.6						
III	0.1	Third	—	4	2	5.5						
IV	0.1*	Third	—	4	9	5.4						
V	1.0	Third	—	4	14	*6.5*‡						

* Plus one-sixth as much pyrethrins.

† Data on first generation rats pertain to both sexes, and include pairs of rats sacrificed at 26 and 52 weeks, all fatalities, and all survivors sacrificed at 104 weeks, except for rats having large tumors or abscesses. Data on second generation rats concern representatives of both sexes from each litter raised to breeding age, and include the one fatality that occurred (in Group II). Data on third generation are for male and female representatives (weanlings) of litters born to females of each second generation litter.

‡ Italicized averages are the only ones significantly different (showing a difference which is four or more times its probable error) from the value on the control group for the same item.

neys were markedly increased in average per cent weight. In the 1.0 per cent group the livers were slightly increased in average per cent weight in all three generations raised on the treated food, and the kidneys were slightly increased in average weight in the first generation only.

Pathology:[2] Because the reaction of the rat to the general effects of disease,

[2] This summary is based on a a concept of normal and abnormal comparative cytology. The manuscript is in preparation by the junior author and includes the citation of the references omitted in this summary.

either induced as in the case of Piperonyl butoxide, or natural, is expressed chiefly by changes in liver, kidney and adrenal, the description and discussion of the effect of ingestion of Piperonyl butoxide on the rat is limited to the effect on these three organs. The liver is most concerned with the reaction to the toxicity of Piperonyl butoxide because all cells are affected by the more severe acute, subacute, and chronic degrees of toxicity of the chemical whereas the proximal convoluted tubules of the kidneys and fascicular zone of the adrenals react more or less selectively to the more severe toxicities. Furthermore, the kidney reacts more than the adrenal in severe chronic toxicity and the adrenals react more than the kidney in severe acute toxicity; the liver reacts severely in each of the toxicities.

Gross findings: The livers were enlarged and discolored pale brownish red to pale grayish brown in the moderate and severe subacute and chronic toxicities. Brownish discoloration was accentuated in the 2.5 per cent group by the accumulation of toxic brown pigment after 41 weeks. The smaller hypodysplastic nodules in the later deaths of the 2.5 per cent group were fairly well defined and grayish white whereas the largest hypodysplastic and largest hyperdysplastic nodules were poorly defined, pale grayish brown and 1.0 to 1.5 cm. in diameter. The two types of nodules occupied more than one-half of the liver in which there was a small primary liver-cell carcinoma; hypodysplastic nodules formed more than 90 per cent of the liver of the last rat to die. The small hepatoma (3 mm.) of the 2-year rat in the control group was grayish white and the larger hepatomas in the 2-year rat of the 1.0 per cent group and next-to-last rat to die in the 2.5 per cent group were yellowish brown and grayish brown respectively. No cirrhosis was recognized; the more severe bile duct changes speckled the cut surfaces with delicate gray flecks. No definite changes were attributable to the focal necroses.

Enlargement of the kidneys in the severe toxicities varied from grayish red in the more acute instances to grayish yellow in the chronic instances. In the acute and several of the early subacute and early chronic deaths the adrenals were red and swollen. They were pale yellow in the later chronic deaths.

Microscopic findings (Table 6): The outstanding toxic reactions of the liver to the general effects of ingested Piperonyl butoxide were dysplasia and dystrophy. Dysplasia was characterized by faulty mitosis which resulted in the formation of diffuse and focal areas and nodules of small haploid cells and large polyploid cells and an increase in binucleate cells in the various categories of ploidy. Dystrophy was characterized by alteration and increase of basic protein and pentose nucleic acid in favorable reactions to toxicity and decrease and disorganization of the substances in unfavorable or exhaustive reactions. Dystrophy included, in addition, hyalin necrosis of cells, cytoplasmic vacuolation, hydropic swelling of the cytoplasm, nucleus and nucleolus, hyalin transformations in the cytoplasm, nucleus and nucleolus, focal necroses, and increase of cell lysis. Inanition, although usually caused by toxicity, was caused also by starvation with little related toxicity and was considered therefore a reaction different from the reaction of toxicity. All categories and varieties of dysplasia were affected by dystrophy and inanition.

Slight changes due to toxicity were found in the control and first three test groups with a very slight equal increase of the changes in the third and fourth groups. The changes were scattered hyalin-necrosis cells, focal and diffuse hydropic swelling and slight dysplasia and dystrophy. In the 1.0 per cent group there were diffuse and advanced hydropic swelling, moderate dysplasia and dystrophy and, in several livers of the two-year sacrificed rats with concomitant natural disease, slight inanition.

TABLE 6

*Summary of type and incidence of microscopic findings in livers, two-year rat experiment on chronic toxicity of Piperonyl Butoxide**

	GROUP NO.					
	I	II	III	IV	V	VI
	Piperonyl. butoxide in food (%)					
	Control	0.01	0.1	0.1†	1.0	2.5
Number of animals	15	12	11	13	7	23
With extrahepatic inflammatory disease	9	4	4	4	3	7
Average per cent weight	3.8	3.5	4.1	3.6	5.9	14.8
Normal	0	0	0	0	0	0
Slight chronic toxicity	15	12	11	13	0	0
Moderate chronic toxicity	0	0	0	0	7	0
Severe chronic toxicity	0	0	0	0	0	23
Slight focal necroses	0	0	0	0	0	8
Moderate focal necroses	0	0	0	0	0	9
Focal hydropic swelling	6	3	2	4	5	0
Diffuse hydropic swelling	3	2	4	4	4	0
Hyperdysplastic nodule	3	0	2	0	0	4
Hypodysplastic nodule	5	5	4	6	5	13
Hepatoma	1	0	0	0	1	1
Hepatic cell carcinoma	0	0	0	0	0	1
Cystic bile ducts	0	0	0	0	0	9
Diffuse lobular cirrhosis	0	0	0	0	0	1
Toxic pigmentation	0	0	0	0	0	15

* For 104-weeks sacrificed rats of Groups I, II, III, IV and V with the exception of one rat in Group V near death from sarcoma, and for rats (all fatalities) of entire group VI.

† Plus one-sixth as much pyrethrins.

Changes in the livers of rats dying of natural disease manifested no recognizable additive toxic effect of Piperonyl butoxide in the first three test groups but did demonstrate additive effect in the 1.0 per cent group. Findings in the livers of the natural fatalities of the 1.0 per cent group approximated those of the earlier deaths of the 2.5 per cent group and the subacute group.

Dystrophy and dysplasia were extreme in the 2.5 per cent group. In the first category of deaths (see Mortality) the reaction of exhaustion, or depletion of reacting substances, was predominant. An inadequate favorable reaction of in-

crease in amount and functional organization of altered reactive substances characterized the second category. In the third category there was an indication of a prolonged favorable reaction which terminally became inadequate. Dystrophy in the third category was elaborated by chronic hyalin changes in the cytoplasm, nucleus and nucleolus, and dysplasia was elaborated by the formation of large nodules of hypodysplastic and hyperdysplastic cells. The reactions of the acute experiment were similar to that of the first category; that is, profound exhaustion, whereas the reaction of the subacute experiment was like that of the second category.

Vacuolation of the cytoplasm and hydropic swelling of the nucleus and nucleolus occurred in all deaths of the acute and subacute experiment and 2.5 per cent group, and in all acute deaths from natural inflammatory diseases. Fat was formed abundantly in the cytoplasm in the livers of animals dying in the acute experiment and of early deaths of the subacute experiment, and was practically absent in the later deaths of the subacute experiment and all deaths in the chronic experiment. Glycogen disappeared nearly completely in the sacrificed rats (7½ hours) of the acute experiment and was absent in the natural and induced deaths. Focal necroses of the various types (coagulative, lytic, hyalin and achromic) were essentially an expression of severe subacute toxicity. They occurred in the subacute experiment, in the 2.5 per cent group and in 6 out of 8 subacute natural deaths in the 1.0 per cent group. Acidophil cells were identified by brightly acidophilic cytoplasm and little nuclear change. The far more common combination of cytoplasmic acidophilia and diffuse nuclear basophilia was identified as hyalin necrosis. Both cytological states indicated toxicity wherever found. Achromic lysis was identified as fading away of the cell with the interphase chromosomes being the last organelles to disappear. An increase in achromic lysis, which was a normal manner of cell death, paralleled an increase in dysplasia and indicated toxicity. Exhaustion necrosis of cells (karyorrhexis) occurred in prolonged severe and induced toxicities in which inanition was severe also. It was characterized chiefly by a slow process of swelling and fusion of the interphase chromosomes with final lysis of the contents of the globules. The process was more rapid and with less swelling and little or no fusion of the chromosomes in the severely acute toxicities. Toxic pigmentation was the ordinary brown pigmentation which occurred characteristically in the cells of the proximal convoluted tubules of the kidney in relation to chronic disease. Its occurrence in the Kupffer and parenchymal cells of the liver indicated severe chronic disease.

Changes similar to those of the liver but less elaborate and less advanced were found in the fascicular zone of the adrenal and proximal convoluted tubules of the kidney. In the most severe reactions the glomerular zone was affected more than the reticular zone of the adrenal.

Inanition exerted a general effect on nearly all cells of the body but was demonstrated best by rounding and severe, homogeneous condensation of the nuclei of the Kupffer cells and the cells of the distal convoluted tubules of the kidney. Inanition occurred in all classes of disease but was invariably present in the chronic natural diseases which also demonstrated changes due to starvation. Deaths

primarily from starvation in the natural diseases showed decrease in cell volume and condensation of the reactive substances of the parenchymal liver cells as well as the reaction of inanition. Starvation in the one rat in the first category of the 2.5 per cent group showed relative decrease in size of the parenchymal liver cells and moderate condensation of reactive substances. The additive effect of natural disease in the 2.5 per cent group on the liver was chiefly that of increased exhaustion and related atrophy.

Dysplastic nodules in liver: Small fairly well circumscribed nodules of cells stood out from the parenchymal diploid cells of the livers in the control and test groups owing to differences in the volume and appearance of the cells of the nodules. Nodules composed of small, haploid cells showed practically no diploid or polyploid cells in the control group but diploidy and polyploidy increased with increase in dosage after the 0.1 per cent group and dystrophy affected the cells as it did the parenchymal cells. Similarly, nodules composed predominantly of tetraploid cells in the control group demonstrated increasing polyploidy which reached extreme degrees in the 2.5 per cent group. The volumes of the cells of each type of nodule increased in proportion to increase in volumes of the parenchymal cells adjacent to the nodules. Small nodules that began with small cells in the control group corresponded with large nodules with large cells in the 2.5 per cent group but the volumes of the cells were less in the categories of ploidy which corresponded to the categories of the parenchymal cells; hence, the term hypodysplastic nodule. Similarly, the categories of ploidy in the nodules in the 2.5 per cent group corresponding to the tetraploid nodules in the control group contained cells larger than the diploid and polyploid parenchymal cells; hence, the term hyperdysplastic nodule.

Appearance of the cells of the nodules differed from the parenchymal cells chiefly because the organization of functional structure of the cells of the nodules was inferior to that of the parenchymal diploid cells. The differences were similar to those of the occasional haploid and polyploid cells which were found normally in livers; that is, the organization of the basic protein-pentose nucleic acid complexes was distorted and deficient. Binucleate cells of the nodules in all categories of ploidy and those normally found in livers were intermediate in apparent efficiency of functional structure. Polynucleate cells were uncommon.

It is noteworthy that the longest lived rats in the 2.5 per cent group were most able to produce dysplastic nodules and life was maintained nearly entirely by the hypodysplastic nodules (liver weighed 30 per cent of body weight of the last rat to die) since the small fraction of parenchyma left was severely altered by toxic reaction and compression by the nodules.

Primary tumors of the liver: The three hepatomas contained practically no normal functional organization of structure. There were no cytoplasmic bodies and no organization of cytoplasmic pentose nucleic acid; basic protein was deficient and pentose nucleic acid was excessive. The cells of each hepatoma were nearly uniform throughout each tumor but differed in the three tumors mainly by the predominant category of ploidy. The hepatomas of the 1.0 per cent and 2.5 per cent groups were composed nearly entirely of diploid cells whereas the

hepatoma of Group I was composed chiefly of tetraploid cells. Polyploidy in the hepatomas was notably inconspicuous as compared to that of the dysplastic nodules but nuclear organization was distorted by the formation of many small nucleoli. No dystrophy was recognized in the hepatomas.

The primary carcinoma was quite pleomorphic and the cells showed extremes of dysplasia but no dystrophy. Deficiency in basic protein and excessive pentose nucleic acid were more pronounced than in the hepatomas. The cells of the metastasis were slightly less pleomorphic than the primary tumor.

Cytoplasmic-nuclear volume ratios, obtained by dividing the cytoplasmic volume by the nuclear volume, were least in the carcinoma and metastases and increased in the following order: hepatoma, hypodysplastic nodule, normal diploid cell, and hyperdysplastic nodule. In other words, the nuclei were relatively largest in the carcinoma and smallest in the hyperdysplastic nodule.

Dystrophy and dysplasia, hyalin-necrosis cells, cytoplasmic vacuolation and hydropic swelling of the nuclei in the cells of the reactive zones of the kidneys and adrenals were generally similar to the slight and moderate degrees of toxic reaction of the liver and were distributed as indicated in the gross description. The other organs demonstrated no significant toxic effects of Piperonyl butoxide except partial destruction of ganglion cells of the brain stem in the acute, and early deaths of the subacute, experiments. Improper development and atrophy of the primary sex organs were associated with stunting and cachexia and occurred only in the 2.5 per cent group.

Evidences of toxicity of Piperonyl butoxide in the livers of the second and third generations of rats in the fourth and fifth groups were similar in nature but slightly increased as compared to those in the sacrificed two-year rats of the first generation. Also, the livers of the rats which were sacrificed at 26 and 52 weeks in the third, fourth, and fifth groups manifested slightly exaggerated findings. The exaggerated findings were attributed to suspected bartonellosis which was more evident in the younger rats, including those of the control group.

Carcinogenicity: The type and incidence of natural disease in the experimental rats (see Morbidity) indicate the common inflammatory loci and the types and locations of the tumors. Omitted from the discussion was bartonellosis which was diagnosed by cuffing of the splenic corpuscles, with proliferating lymphocytes, and by the invariably associated slight or moderate peribronchial infiltration with plasma cells and lymphocytes; plasma cell infiltration of lymph nodes was severe. No definite relation existed between prominence of inflammatory loci and the occurrence of tumors with the exception, perhaps, of the occurrence of two sarcomas, out of the five, as primary lymphoid sarcomas of the lungs. The incidence of 5 sarcomas in 93 second-year rats indicated that the strain of rats possessed a definite, but slight tendency to develop malignant tumors. Inasmuch as one sarcoma occurred in each of the first five groups (including the controls), and one of the lymphoid sarcomas occurred in the control group, there was no evidence that Piperonyl butoxide possesses the undesirable property of stimulating the formation of malignant tumors. The occurrence and types of benign tumors in the test groups were similar to those of the control group. The maintenance of the extreme dystrophy and dysplasia throughout the 68 weeks in the 2.5 per cent rat

group with the occurrence of only one carcinoma (and one hepatoma in the same rat), at 64 weeks, clearly demonstrates that Piperonyl butoxide is without significant carcinogenicity as related to the liver.

TABLE 7

Data on dogs and goats, one-year chronic toxicity test of Piperonyl Butoxide

GROUP NO.	DOSAGE OF PIPERONYL BUTOXIDE	ANIMAL NO.	SEX	AGE AT START OF TEST	INITIAL WEIGHT	TOTAL AMT. PIPERONYL BUTOXIDE RECEIVED	CHANGE IN WEIGHT WHILE ON TEST
	ml./kg./day			*weeks*	*kg.*	*ml.*	%
I	0	Dog 12	F	35	14.6	0	+9
	0	Dog 22	M	15	4.4	0	+93
	0	Dog 32	M	40	9.4	0	+6
	0	Dog 38	F	36	7.8	0	+32
II	.002–.003	Dog 16	M	35	19.2	15.4	+4
	.003	Dog 25	M	15	3.2	7.3	+169
	.003	Dog 36	F	40	6.9	8.3	+41
	.003	Dog 42	F	36	8.4	9.4	+17
III	.02–.03	Dog 15	F	35	16.0	139.1	−1
	.03	Dog 21	F	23	5.5	67.8	+27
	.03	Dog 24	M	15	3.1	2.3	+13
	.03	Dog 35	M	40	7.9	84.0	+29
IV	.14–.16	Dog 26	M	52(?)	12.5	530.4	−6
	.1	Dog 34	M	40	7.7	199.4	−19
	.1	Dog 40	F	36	7.9	216.3	−10
	.1	Dog 41	F	36	8.4	240.1	−8
V	.2–.7	Dog 13	F	35	18.1	261.1†	−62
	.3	Dog 23	M	15	4.1	24.3	−44
	.3	Dog 33	M	40	9.8	141.9	−36
	.3	Dog 39	M	36	7.5	180.9	−39
	.053–.065	Goat 3	F	104(?)	30.6	624.0	+23
	‡	Goat 8	F	4/7	2.5	‡	+1080

* Deaths occurred as follows: Group III—Dog 24 at 3-3/7 weeks; Group V—Dog 13 at 21 weeks; Dog 23 at 4-3/7 weeks, Dog 33 at 9 weeks, Dog 39 at 15 weeks. All other animals survived throughout the period of the test (52 weeks).

† In two series of doses separated by an eleven-week rest period.

‡ Not treated, but being nursed by treated dam (Goat 3) from 4 days of age for one-half year.

II. Chronic toxicity experiments on dogs and goats

Dogs (Table 7): The results of the experiments on chronic toxicity ion these larger animals were characterized by a relatively simple toxic reaction to Piperonyl butoxide fundamentally similar to that demonstrated in the rat. The changes in the third, fourth, and fifth dog groups corresponded remarkably closely to the changes in the 0.1 per cent and 1.0 per cent groups and in the first category of

the 2.5 per cent group, respectively, of the rat. Furthermore, the symptoms of toxicity in the fourth and fifth dog groups were similar to those observed in the 1.0 per cent and 2.5 per cent rat groups respectively. There was no evidence of toxic reaction to the chemical in the second group.

One natural death occurred, in the third group, in a previously healthy 18-week old male dog which died four days after the onset of an acute illness and 24 days after the beginning of the experiment. The immediate cause of death was ordinary severe central and midzonal acute necroses, or atrophy of the liver of undetermined etiology. Morbidity from natural disease was practically nonexistent. All dogs in the fifth group died of liver damage caused by Piperonyl butoxide.

Dog 13, as indicated in Table 7, fifth group, was treated over two separate periods of time with complete biologic recovery from the first treatment during the intervening 11 weeks. No evidence of previous liver damage was recognized and the microscopic appearance was quite like that of each of the other dogs in the group.

Dog 26, fourth group, which received an enormous total dosage (530 ml.) of Piperonyl butoxide without apparent effect except slight weight loss, successfully passed a breeding test at the first offering after 42 weeks on the experiment; a litter of normal pups was sired.

Red and white blood cell counts were without significant deviation from the normal in all groups of dogs.

The average per cent of body weight for the livers in each group in the order listed in Table 7 was 2.5, 2.9, 3.2, 3.5, and 5.1. No gross changes of the liver were noted other than brownish discoloration in the fourth group and dark reddish brown discoloration in the fifth group. The adrenals were increased slightly in per cent of body weight (0.03) in the fourth group without changes in appearance; the percentage weight (0.04) was doubled in the fifth group, and the inner halves of the cortices, which were the reactive zones in the dog, were swollen and dark red. The kidneys were enlarged in the fourth and fifth groups (0.6% and 1.0% of body weight respectively) and the cortices were swollen and brownish.

Microscopic findings, as indicated above, were more simple but quite comparable to those of the corresponding rat groups. Hydropic swelling was most conspicuous in the third group, dystrophy and dysplasia became more prominent in the fourth group and dominated the changes in the fifth group. Acidophil and hyalin-necrosis cells increased in number as the dystrophy increased. No focal necroses occurred but there was diffuse pericentral achromic lysis in the fifth group. The extreme dysplasia with the severe polyploidy and the formation of nodules of the later deaths in the 2.5 per cent rat group did not occur in the dog; neither did the hepatomas and carcinoma.

Goats (Table 7): Goat 3 was fed a total of 624 ml. of Piperonyl butoxide over a period of one year. During this period it increased 22.9 per cent in body weight and nursed a female kid (Goat 8) very adequately for 27 weeks. The general health of both goats was good and no clinical evidence of toxicity appeared in either one. Terminal blood cell counts revealed no evidence of leucocytosis or anemia. Microscopic examination of the liver of the dam showed slight dystrophy

and dysplasia and central hydropic swelling with slight fatty accumulation; the other organs were negative. These findings were considered as evidence of subbiologic toxicity. The examination of the organs of the kid was negative.

III. Short term experiment on monkeys

To secure additional evidence of species variation in susceptibility to continued ingestion of Piperonyl butoxide, and in order to determine if the same symptoms (especially anorexia) would occur in primates as in rats and dogs, a four week toxicity test was made on monkeys. No symptoms whatsoever developed in two African Green Monkeys fed Piperonyl butoxide by capsule six days a week for four weeks at the two intermediate dosage rates used in the dog experiment (0.03 and 0.1 ml./kg./day). The actual sizes of the daily doses of Piperonyl butoxide fed to the two monkeys were 0.03–0.04 ml. and 0.14 ml. respectively, and the total amounts of the insecticide fed to these relatively small animals during the experiment were respectively 0.94 and 3.36 ml. The higher dosage rate had been found to represent approximately the toxicity threshold for dogs, in which it caused some anorexia, vomiting, and slight loss of weight, but it had no effect upon the monkey.

No gross changes were recognized in the livers except for increase in size of the liver of the control monkey; it weighed 4.19 per cent of body weight as compared to 2.13 per cent in the 0.1 ml./kg./day monkey and 2.7 per cent in the 0.03 ml./kg./day monkey. The excessive weight of the liver of the control monkey was attributed to a reaction to toxicity of natural disease of the lung which contained scattered small areas of pneumonia.

Microscopic findings of minimal dystrophy and dysplasia, occasional acidophil and hyalin-necrosis cells and hydropic swelling of the 0.1 ml./kg./day monkey were quite comparable to the findings of the 0.03 ml./kg./day group, or third group of the dogs. The liver of the 0.03 ml./kg./day monkey showed no evidence of toxic reaction to Piperonyl butoxide. Adrenals and kidneys were practically normal in the test animals. The decrease of reaction of the monkey as compared to the dog is due, perhaps, to the short time of the experiment as compared to the long dog experiment.

IV. Biochemical balance experiments

In each experiment (see item 6; Experiments) practically all of the Piperonyl butoxide was eliminated quickly in the feces; the urine contained little more than trace amounts. There was a recovery from feces of 6550 mg. in the first 48 hours as compared to 38 mg. in the second 48 hours of the first experiment, and 6696 mg. in the first 48 hours as compared to 47.9 mg. in the second 48 hours in the second experiment. The total amount recovered from the urine was 0.23 mg. in the first experiment and 7.0 mg. in the second. No Piperonyl butoxide was recovered in the feces and urine after 48 hours in the second experiment. The total amounts recovered, from feces, urine and pan washings, was 6590.56 mg. (78.0 per cent) in the first experiment and 6750.9 mg. (87.6 per cent) in the second experiment. No symptoms occurred in either experiment.

DISCUSSION

Our previous toxicological studies (Sarles, *et al.*, 1949) dealt mainly with acute oral toxicity and skin and eye irritation. They indicated that Piperonyl butoxide, both by itself and combined with pyrethrins in the concentrations used in practical formulations, is relatively nontoxic and nonirritating. These findings are in general agreement with the limited data on Piperonyl butoxide in the literature (Lehman, 1948, 1949; Draize, *et al.*, 1948).

The present results deal mainly with studies of effects of chronic toxicity from long continued ingestion of Piperonyl butoxide, both alone and in combination with pyrethrins, and furnish data upon which it appears feasible to estimate the amount of these insecticides which can be safely ingested daily by humans without danger of harmful effects. The evaluation of the chronic oral toxicity of Piperonyl butoxide for rats and dogs has been made on the basis of the outstanding biologic findings concerning rate of growth, weight loss, symptoms of toxicity, reproductive ability and mortality, and the outstanding microscopic findings of dystrophy and dysplasia in the livers, kidneys and adrenals of the animals. The graded chronic experiments on the rats and dogs were comparable within reasonable accuracy with respect to these two criteria. If the approximate equivalent values of 80, 700, 3000, and 11,000 parts per million of Piperonyl butoxide in the dog rations are substituted for the actual dosage rates by capsule the results in the two species can be directly compared. Hence the approximate parts per million of Piperonyl butoxide in the rations of rats and dogs respectively which were without any biologic and pathologic toxic effect were 100 and 80; the amounts which were tolerated in good health were 1000 and 700; the amounts which were tolerated with moderate impairment of health were 10,000 and 3,000; and the amounts which represented a fatal dosage were 25,000 and 11,000.

An estimation of the daily dose of Piperonyl butoxide which could be ingested safely by humans has been calculated from the data on Group IV of the second two-year rat experiment. The rats of this group ingested a ration containing a high level of Piperonyl butoxide (0.1% = 1000 p.p.m.) plus one-sixth as much pyrethrins for two years without showing biologic toxicity. (In the following calculation of the safe daily dosage for man, Piperonyl butoxide only will be considered, both in order to simplify the discussion, and because it was found that adding one-sixth as much pyrethrins to the Piperonyl butoxide did not produce symptoms of biologic toxicity; the comparable figures for pyrethrins may be derived from the figures for Piperonyl butoxide by dividing by six). The daily dose of Piperonyl butoxide consumed without harm by these rats has been calculated from their average food consumption (23 grams of food/day/rat) and their average body weight (273 grams) in the 26th week of the experiment. The data at this interval were utilized for the calculation because the food consumption and the body weight of the rats had nearly reached their maximum values by the 26th week, and remained at about this level for the major part of the experiment. The daily dose of Piperonyl butoxide ingested safely by these rats was 84 mg. of Piperonyl butoxide/kg. of rat/day. This dosage rate would be equivalent to a daily dose of 4200 mg. of Piperonyl butoxide for an average 110 pound (50 kg.)

person. If this value is divided by 100 in order to allow both for an extremely conservative safety factor and for differences in species susceptibility (although the latter appear to be slight in the case of Piperonyl butoxide), the safe daily dose for an average 110 pound person is calculated to be 42 mg. of Piperonyl butoxide. Assuming that an average person eats 1000 grams (1,000,000 mg.) of food a day, this amount would represent a concentration of 42 parts per million in the food. Inasmuch as only a very small part of the total food eaten would ever conceivably contain any Piperonyl butoxide, the number of parts per million which could safely be ingested on any one item of food would be several times this amount.

Actual toxicity of humans through the possible contamination of food and foodstuffs by Piperonyl butoxide is most unlikely owing to the extremely small amounts required for insecticidal effect. For example, protection for long periods of time against insects infesting grain has been obtained with treatments which result in a Piperonyl butoxide content of 13 to 15 p.p.m. in the whole grain, much or all of which is removed in the cleaning process before milling of the grain. Forty-two parts per million of Piperonyl butoxide in the overall diet of man would represent a massive contamination of a single dietary item.

The active principles of pyrethrum, the pyrethrins, are of a low order of toxicity to warm-blooded animals (Lehman 1948, 1949), and for this reason they enjoy an old and established use in connection with food and foodstuffs. As already mentioned, Piperonyl butoxide is synergistic with pyrethrins as regards insecticidal properties, and this permits economy in the use of the expensive pyrethrins. The chronic toxicity test on rats in Group IV shows, however, that the heightened insecticidal action of this combination is not accompanied by an increased toxicity to warm-blooded animals. Results paralleled those obtained with Piperonyl butoxide alone, showing the same low order of pathologic toxicological action and absence of biologic toxicological action for the combination.

The absence of any synergistic effect on animals from the combination may be explained in part by the very small proportion of Piperonyl butoxide which is absorbed from the alimentary tract, as demonstrated in the Biochemical Balance Experiments, and partly by the presumed inactivation of pyrethrins in the alimentary tract (see review of literature on the toxicity of pyrethrins in Gnadinger (1936, 1945) and comments of Sheppard (1939).

SUMMARY AND CONCLUSIONS

1. The biologic and pathologic effects of Piperonyl butoxide, a synthetic organic insecticidal material used synergistically with pyrethrins, were studied by an acute toxicity test on rats, by subacute toxicity tests on rats, by graded-dosage chronic toxicity tests on rats and dogs, by a low-dosage chronic test on a goat and by a short term experiment of low and moderate dosages on monkeys.

2. Piperonyl butoxide was found to be a material of a relatively low order of toxicity for the tested warm-blooded animals. There was relatively little difference between sexes and between species of animals (rats, dogs, monkeys, goat) in susceptibility to this material, and no indication of cumulative toxic effect upon

the second and third generations of rats fed food containing up to 10,000 parts per million of Piperonyl butoxide.

3. The outstanding biologic findings of moderate toxicity caused by chronic ingestion of relatively large doses of Piperonyl butoxide (10,000 and 25,000 p.p.m.) were anorexia, wasting, reduced food consumption, reduced or lost ability to reproduce and accentuation of concomitant natural disease.

4. Biologic and pathologic findings in a two-year, graded dosage, paired-rat experiment, in which Piperonyl butoxide was added to a standard diet and fed *ad libitum*, indicated the following degrees of toxicity:

a) 100 p.p.m. were nontoxic.

b) 1000 p.p.m. were tolerated in good health.

c) The addition of one-sixth as much pyrethrins to a ration containing 1000 p.p.m. Piperonyl butoxide produced no additional toxicity.

d) 10,000 p.p.m. were tolerated with moderate toxicity.

e) 25,000 p.p.m. were fatal to a group of 24 rats during a period of 4 to 68 weeks.

5. Biologic and pathologic findings in a one-year graded-dosage dog experiment, in which Piperonyl butoxide was fed six times a week by capsule, indicated the following converted degrees of toxicity:

a) Approximately 80 p.p.m. were nontoxic.

b) Approximately 700 p.p.m. were tolerated in good health.

c) Approximately 3000 p.p.m. were tolerated with moderate toxic effect.

d) Approximately 11,000 p.p.m. were fatal in 4 to 15 weeks.

6. Subbiologic toxic lesions observed in the livers of rat, dog, goat and monkey in good health were acidophil and hyalin-necrosis cells, hydropic swelling and slight dystrophy and dysplasia. These lesions were nonspecific and no significant change occurred in incidence and degree, as compared to lesions in the livers of the control rats and dogs.

7. In moderate biologic toxicity, the outstanding lesions of rat and dog were moderate numbers of acidophil and hyalin-necrosis cells, moderate dystrophy and dysplasia and advanced hydropic swelling; the lesions were accentuated in instances of concomitant natural disease.

8. Biochemical Balance studies on a dog indicated that when a large dose of Piperonyl butoxide is fed by stomach-tube it is quickly eliminated in the feces, and that only traces appear in the urine.

9. Piperonyl butoxide was not carcinogenic for the liver. It did not exert a malignant tumorogenic effect upon the general tissues, nor upon the endocrine glands and breasts, and did not have an indirect general tumorogenic effect through disturbance of the endocrine glands.

10. The safe human tolerance for chronic ingestion of Piperonyl butoxide was estimated, after allowing for a 100-fold safety factor, to be 42 parts per million in all the food eaten.

ACKNOWLEDGMENTS

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Chicago, Illinois, November 15–17, 1951

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Charles Franklin Craig Lecture: L. W. Hackett, M. H. Soule, and T. T. Mackie
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MINUTES OF THE ANNUAL COUNCIL MEETING

The annual dinner and business meeting of the officers and councilors was held November 14, 1951, at the Congress Hotel in Chicago, Illinois. Officers and councilors present were: Paul F. Russell, Harry Most, Quentin M. Geiman, Lewis W. Hackett, Marshall Hertig, James N. DeLamater, G. Robert Coatney, Donald L. Augustine, George M. Saunders, Tom F. Whayne, and Herbert G. Johnstone. Past-presidents present were: George C. Shattuck, Louis L. Williams, Jr., George R. Callender, Herbert C. Clark, Edward I. Salisbury, and E. Carroll Faust. Samuel W. Simmons represented the National Malaria Society. The president of the Society, Paul F. Russell, presided.

The minutes of the previous meeting held November 7, 1950, at the Hotel De Soto in Savannah, Georgia, were approved as published in the AMERICAN JOURNAL OF TROPICAL MEDICINE of May, 1951.

The Secretary's report was presented as follows:

The present report covers the period from November 1, 1950, to October 31, 1951. Activities during the year can be divided primarily into three categories: (1) routine duties, (2) amalgamation proceedings in collaboration with the National Malaria Society, and (3) planning and arranging the program for the annual meeting. This report deals only with the first category.

Active membership in the Society for the past year was slightly above the level achieved last year. There are 861 members in good standing at the present time, of which 66 are new members. There are 18 honorary members and 1 emeritus member; 86 members are delinquent in dues, of which 63 have been delinquent for one year and 23 for two years; 49 members were delinquent in dues for three years and were automatically dropped from membership on October 1; 13 members were lost by death and 34 by resignation; 2 resignations are pending action on their nomination for emeritus membership. The total membership now stands at 966, one less than the total membership of 1950.

The Secretary announces with regret the loss by death of three honorary and ten active members:

E. Brumpt, Honorary Member
C. F. Craig, Honorary Member, Former President (1914–15) and Former Editor of AJTM
G. Dock, Honorary Member
M. A. Azim
B. B. Morgan
C. A. Powell
H. O. Proske
A. C. Reed
W. A. Sawyer
R. A. Sloan
M. H. Soule
W. H. Spruell
J. D. Weis

Resignations:

E. B. Agnor
H. Beckman
C. W. Berry
M. F. Boyd
M. Brandsma
M. H. Brodkey
J. M. Carlisle
A. Cornejo
D. B. Cowie
E. F. Dombrowsky
R. C. Dunn
W. F. Eisenstaedt
H. Finkelstein
A. A. Goldbloom
H. M. Horack
G. K. Ingham
E. R. Kadison
A. M. Kasich
H. A. Kemp
J. R. Matson
W. C. McCarty
D. F. Milam
G. McF. Mood
H. D. Moor
L. Pearce
J. F. Perkins
R. J. Pickard
D. L. Rodgers
S. Ross
J. L. Tullis
C. J. Van Slyke
D. W. Walker
R. L. White
R. L. Wyatt

The following persons have been elected to membership:

F. R. Abinanti
M. S. Ambrose
J. C. Andersen
H. C. Barnett
P. N. Bhatt
M. Bloch
J. F. Busey
C. M. Cameron
C. M. Carpenter
S. E. Castro
H. T. Chaghlassian
R. Chappell
F. S. Cheever
W. P. Clavecilla
B. A. Cole
H. C. Conn
A. N. Contopoulos
E. J. deBeer
S. A. Edgar
R. W. Edwards
A. L. Ejercito
R. Elsdon-Dew
D. E. Eyles
D. E. Fader
D. H. Feldman
R. C. Fish
M. W. Freymann
S. E. Gage
H. H. Gray
H. E. Hamilton
H. V. Herndon
W. J. Hoke
S. M. K. Hu
T. C. Johnson
M. A. Kader-M
F. T. Kerrigon
H. W. Keschner
A. J. Khoury
W. Konigsberger
G. H. Laing
J. F. Lawlis
A. LePoole
J. R. McMillan
K. L. Mallik
C. W. Mangun
A. R. Mead
G. Meiklejohn
M. A. Mills
H. W. Mitchell
C. M. Mofidi
C. S. Richards
R. E. Ryckman
C. R. Schneider
N. S. Scrimshaw
D. Snidvongs
M. A. Stirewalt
L. Szyfman
E. J. Talbott
I. B. Tarshis
H. Turkel
V. M. Villarejos
E. R. Ware
T. Waters
A. B. Weathersby
S. F. Wood
R. A. Wortham

Letters to Officers and Councilors: Three letters were mailed during the year for expressions of opinion and votes. In March, a letter was sent requesting opinions and votes on an application to the A.F.T.M. for $2,000 to be used for publishing extra pages in the Journal; on the publication of a memorial issue of the A.J.T.M. in honor of Col. C. F. Craig; about the sum to be contributed to the cost of a bust of General Gorgas for the Hall of Fame; about titles for a symposium or panel discussion for the scientific program of the annual meeting; and about increasing the registration fee at the annual meeting from $1.00 to $2.50 to help finance invited speakers.

The officers and councilors unanimously approved an application to the A.F.T.M. for funds. The publication of a memorial issue to honor Colonel Craig was approved if no additional financial obligations were entailed. Contributions

suggested for the bust of General Gorgas averaged out to approximately $100, and a check for that sum was sent to the Hall of Fame Committee. A panel discussion for the annual meeting entitled "Tropical Medicine in the Armed Forces" was approved, and raising the registration fee at the annual meeting to $2.50 was approved to help finance invited speakers.

In May, a semi-final copy of a new constitution and by-laws drafted by the Joint Amalgamation Committee was submitted for suggestions and votes; permission was asked to spend the sum of $100 to obtain legal advice for the amalgamation proceedings with the National Malaria Society. At this same time, the officers and councilors were notified that the resignation of Dr. Mark Boyd was not accepted and were asked to vote on the nomination of Dr. Mark Boyd as an honorary member of the Society. The letter also contained the news that the A.F.T.M. had granted the sum of $750 to the Society to publish extra pages in the Journal and information about plans for the annual meeting.

The proposed constitution and by-laws for the amalgamated society was approved for circulation to members for their votes, and the sum of $100 was voted to finance legal advice for amalgamation. The election of Dr. Boyd as an honorary member was not approved.

The final letter in October contained the plans for the annual meeting, a request for items to be placed on the agenda of the business meeting, and statements about invitations to the Society for a meeting place in 1952.

Additional activities concerning the committees will be presented under their respective headings in the agenda.

The Addressograph machine authorized for purchase last year was not bought. Since the amalgamation proceedings looked very promising last year, postponement of investment in a new machine and stencils until after amalgamation was thought to be a good policy.

Recommendations: (1) Since the amalgamation of this Society with the National Malaria Society is scheduled to take place in the near future, permission to purchase a new Addressograph machine for use by the Secretary will be requested.

(2) It is further recommended that the Secretary-Treasurer be allotted a sum not to exceed $110 per month for secretarial assistance during the coming year.

Finally, I wish to acknowledge the splendid cooperation of the officers, councilors, and committees in transacting the business of the Society during the year. In particular, I wish to acknowledge the close relationship with the officers of the National Malaria Society in helping to organize the amalgamation proceedings.

Respectfully submitted,
Quentin M. Geiman.

The report of the Secretary was accepted as read.

As a result of recommendations made by the Secretary-Treasurer, the purchase of a new Addressograph machine was authorized, and funds were voted for secretarial assistance not to exceed the sum of $110 per month. A motion was passed

authorizing the Secretary-Treasurer to engage the services of a certified public accountant to close out the accounts of the Society because of amalgamation and to aid in setting up an accounting system in the new amalgamated society.

The report of the Editor of the AMERICAN JOURNAL OF TROPICAL MEDICINE was presented as follows:

This report has to be made each year before the November issue has appeared and, therefore, the cost data on the current volume are not available. The financial report for Volume 30 (1950) prepared from data submitted by the publishers, The Williams & Wilkins Company, is presented in the following table together with the figures for Volume 29 for comparison.

FINANCIAL STATEMENT FOR VOLUMES 29 AND 30

	Vol. 29 (1949)	*Vol. 30 (1950)*
Income:		
Paid subscriptions	$ 9,180.96	$10,534.64
Advertising	1,079.75	764.70
Paid by authors	2,104.60	2,822.61
Paid by ASTM	3,880.24	1,560.00
Net from reprint sales	77.07	14.18
Back volume sales	495.12	685.71
Total	$16,817.74	$16,381.84
Production cost:		
Manufacturing	11,323.13	10,353.12
Distribution, overhead, etc.	2,216.24	2,789.71
Total	$13,539.37	$13,142.83
Surplus	3,278.37	3,239.01
Accruing to ASTM (50%)	$ 1,639.18	$ 1,619.51
Paid subscriptions:		
Members	687	850
Nonmembers	1,012	1,191
Total	1,699	2,041
Not renewed: Members	122	
Nonmembers	528	
Total not renewed	650	

The Society was credited with the sum of $1,619.51 as its share of the net surplus for the year 1950 and appropriated $1,560.00 to pay for 130 pages published in 1950 (Volume 30) in excess of the contractual number of 500. Of this sum, $1,000 had been voted for extra pages in 1950 and the rest, or $560, was taken from the appropriation of $1,500 voted for extra pages in 1951. This action reduced the amount available in 1951 to $840, sufficient to purchase about 73 extra pages. Since the new Editor found himself at the beginning of the year with a considerable number of accepted manuscripts on hand (58 in fact, or enough al-

most to fill four numbers), he appealed, with the approval of the President and Council, to the American Foundation for Tropical Medicine for help; and that organization responded with a generous subsidy of $750, thus providing an additional 58 pages or so. The total number of pages available for Volume 31 (1951) was thus boosted to 631, or about 105 pages for each of the 6 issues. This volume, to be completed by the November number, is expected to run to 887 pages, an average of 148 pages per issue. The excess is provided by authors' subsidies which are obviously indispensable to the production of a journal of satisfactory size and coverage. The expense of publishing the Journal in 1951 is not expected therefore to exceed the funds provided, although the cost of printing has gone up from $10.66 per page in 1949 to $12.00 in 1950 and about $13.00 in 1951.

The present Editor, on assuming his position, inherited 58 papers, submitted and accepted in 1950 but not yet published, and received 97 more through October, 1951, a total of 155 manuscripts, of which 31 were declined or withdrawn. Volume 31, however, when complete, will contain 101 articles. It is evident that rather drastic measures have had to be instituted to bring the material within the capacity of the Journal, in order not to incur a deficit. This has been achieved in three ways: by tightening the editorial policy with respect to acceptance of papers; by trying to obtain from authors the maximum condensation of material compatible with a satisfactory presentation; and by pressing authors to secure subsidies from their institutions or other sources to pay all or part of the cost of publication of the paper. By these means, the papers rejected or withdrawn rose from 12 per cent in 1950 to 32 per cent in 1951, which has seemed to some authors a slight excess of zeal, while the average length of published articles was reduced from about 9 pages to 8 pages, a total reduction of 100 pages for the volume, accomplished I believe without damage and probably with some benefit to the interest and effectiveness of the presentations. Unfortunately, the number of authors or institutions sponsoring the cost of publication remained about the same, 22 of the 101 contributors. In the matter of rejection or revision of manuscripts, the Editorial Board has been of immense service to the Editor, and I wish to take this public opportunity to thank each member for his conscientious, prompt, and competent judgment of the manuscripts sent to him for appraisal.

The disposition of the papers submitted for publication in 1950 and through October, 1951, is shown in the following table:

Manuscripts	*Vol. 30 (1950)*	*Vol. 31 (10 mos., 1951)*
From previous year	50	58
Submitted during year	119	97*
Declined	11	25
Withdrawn	3	6
Accepted	105	66
Available for publication	155	124
Published during year	97	101
On hand for next volume	58	23

* Through October, 1951.

The type of papers submitted in 1951 is shown below:

Subject	*Submitted (To Oct. 31, 1951)*	*Accepted for Publication*	*Field of the Accepted Papers*	*No.*
Protozoa	30	21	Experimental	30
Helminths	23	11	Clinical	14
Virus & Rickettsias	11	11	Epidemiology, Prevention & Control	12
Bacteria & Fungi	12	9	Nutrition	1
Entomological	10	5	Taxonomy	3
Others	11	9	Others	6
Total	97	66		66

In 1951, of the 66 papers accepted, 44 were published at the expense of the Society, while 22 were subventioned by the authors or their institutions to cover all or part of the cost of printing. In this way, 277 extra pages were purchased, for a total of $2,557.70.

In view of the project to fuse the National Malaria Society and the American Society of Tropical Medicine next year and combine their journals, no recommendations can be made at this time for financing the resulting publication. However, the Editor soon discovered that it was impossible without secretarial help to indulge in the correspondence necessary to raise the quality of our Journal, and he found it necessary to disburse considerably more than the $200 appropriated by the Society to cover his office and incidental expenses. With a larger journal in prospect next year, he would recommend that provision be made for a reasonable amount of secretarial assistance to the Editor.

Respectfully submitted,
L. W. Hackett, Editor

The Editor's report was accepted as read.

The Editor was requested to submit a bill covering the added expense of his office incurred during the past year, and a motion was voted to reimburse the Editor for that amount. The Editor was assured that a satisfactory budget for the operation of his office would be approved by the amalgamated society.

The report of the Editor of TROPICAL MEDICINE NEWS was presented as follows:

During the year, the mailing list of TROPICAL MEDICINE NEWS remained at approximately 2000. The maximum printing was for the August issue, 2262 copies. The lowest was February, with 2039. Increase in distribution is largely attributable to additions to the nonmember mailing list.

We are indebted to Eli Lilly and Company, Lederle Laboratories, Merck and Sons, Winthrop-Stearns, Charles Pfizer and Co., and Appleton-Century-Crofts for their financial support.

No supplement was issued in 1951. Type of "The Diagnosis of Leprosy" was destroyed, but we are still holding type of "Health Hints for the Tropics." There was one reprinting of 1000 of the latter supplement, to satisfy a continuing demand. Part of the cost of reprinting was defrayed by sale of 300 copies to the

Economic Cooperation Administration. The revision of "Health Hints" is now in manuscript, and it is anticipated that a considerable portion of the standing type will be usable; hence, the printer has been instructed to continue to hold it.

A change of format was made, starting with the April issue. This consisted mainly of modifying headings of sections and items. A number of letters expressing approval of the change have been received. It is hoped that the Council will consider, further, a change in the cover design.

The schedule for printing the News has also been improved. Whereas, in 1950 there was a routine delay of six weeks between the preparation of copy and publication, the schedule in 1951 was arranged on the basis of a three-week interval between copy and printing. This has been beneficial in enabling us to bring the issues more up to date. For example, it will be possible for us now to insert the news of the annual meeting in the December issue. Under the old schedule, this would not have been possible.

The annual financial statement will be published in the News early in 1952. While we are still operating profitably this year, increased costs of publication without increased returns from advertising threaten to decrease the rate of return the News has previously enjoyed. Also, for the last two years the News has been distributed to the members of the American Foundation for Tropical Medicine, and part of the cost of printing and mailing has been borne by the Foundation. There is some doubt that this support will be continued, although withdrawal is not yet definite. In order to compensate for increased costs, advertising has been solicited for one more page per issue for the coming year.

The Editors wish to thank the Society members for their contributions of newsworthy items. It is hoped that a larger number of notices can be received in the future.

FINANCIAL STATEMENT

TROPICAL MEDICINE NEWS	*January 1, 1951, to October 31, 1951*
Balance in bank, January 1, 1951	$1,349.84
Deposits in 1951	
From 1950 ads	375.00
From AFTM 1950 and 1951	422.97
From 1951 ads	1,500.00
Miscellaneous receipts (sale of supplements)	26.41
Total receipts	$3,674.22
Disbursements	
Editorial assistance	$ 300.00
Printing of TMN	1,315.72
To Society	520.00
Office expense	20.00
Total disbursements	$2,155.72
Balance in bank October 31, 1951	$1,518.50

The Auditing Committee, composed of G. Robert Coatney and George M Saunders, reported that they had examined the financial statement of TMN. Since no records were submitted, the financial statement was not audited.

After a brief discussion, the Council voted to accept the report as read. The Treasurer's report was presented as follows:

FINANCIAL STATEMENT, OCTOBER 31, 1951

Cash and stamps on hand, October 31, 1950	$ 12.93	
Bank balance on Oct. 31, 1950	2,839.17	
		$ 2,852.10
Checking account		
Dues and subscriptions from Nov. 1, 1950 to Oct. 31, 1951	5,452.50	
Receipts from 1950 meeting account	74.12	
Receipts from commercial exhibits	213.40	
Royalty from Journal, 1950	1,619.51	
From Tropical Medicine News, 1951	520.00	
From A.F.T.M. for extra pages in Journal, 1951	750.00	
Fund for hospitality session, Julius Hyman Co.	400.00	
		$ 9,029.53
		$11,881.63
Disbursements		
Subscriptions for Journal	$3,604.00	
Stationery, printing and supplies	265.19	
Stamps and postage	168.79	
Secretarial help	1,188.15	
Travel	210.86	
Bank charges	9.63	
Extra pages, Journal	1,560.00	
Meeting Fund, 1951	100.00	
Gorgas Hall of Fame Contribution	100.00	
Amebiasis Committee	46.91	
Amalgamation (Legal fee, copy of charter)	28.00	
To Editor of Journal	200.00	
Fund for hospitality session, Julius Hyman Co.	400.00	
		$ 7,881.53
Balance		$ 4,000.10
Assets and Liabilities		
Assets		
Bank account balance	$4,549.10	
War Bonds, Series F, purchase price	6,512.00	
Cash and stamps on hand	18.93	
		$11,080.03
Liabilities		
Checks outstanding	$ 549.00	
Fund from A.F.T.M. for Journal	750.00	
		$ 1,299.00
Total Assets		$ 9,781.03

Journal account		
In account Oct. 31, 1950	$ 0.00	
Voted from Treasury for 1951	1,500.00	
Received from A.F.T.M. for extra pages	750.00	
Total		$ 2,250.00
Disbursements		
Deficit for extra pages in Journal, 1950		560.00
Balance available for Journal, 1951		$ 1,690.00

Quentin M. Geiman, Secretary-Treasurer

Councilors G. Robert Coatney and George M. Saunders, who were appointed by the President to audit the Treasurer's books, approved the annual financial statement.

The Council voted to accept the report as read.

Reports of Committees:

Membership Committee—submitted by Gilbert Otto.

During the past year, the Membership Committee considered and approved 66 applications for membership. Of these, 57 were from the United States and 9 from abroad, representing 8 countries.

Questions and points of information were raised concerning several candidates, but none were rejected.

Circular letters were sent to 40 members who were two to three years in arrears inviting reaffiliation with the Society. The results were the payment of back and current dues by 2 members and a resignation from 1; 37 did not respond at all.

This committee offered recommendations, through the Secretary, to the Constitution Committee of the proposed American Society of Tropical Medicine and Hygiene. Our principal recommendations are that active membership not be restricted, by actual statement or ambiguity of expression, to U.S.A. citizens or to citizens of the Americas; that honorary membership be open to all candidates regardless of nationality; and that the procedure for election of active members be simplified.

Honorary Membership Committee—no report was received.

Charles Franklin Craig Lecture Committee—By a unanimous vote, C. C. Bass was elected to deliver the sixteenth annual lecture. As an intimate friend of Colonel Craig, Dr. Bass consented to deliver the lecture under the title "Charles Franklin Craig—Fifty Years of Work and Service in Tropical Medicine."

C. F. Craig Memorial Issue—The report of this committee composed of W. A. Sodeman, L. W. Hackett, G. R. Callender, and J. S. Simmons was made by Dr. Hackett. The organization of the Memorial Issue was reported as nearing completion, and plans include its publication as Issue No. 1 of the Journal for the coming year.

Walter Reed Medal—no medal was awarded this year.

Program Committee—presented by the Chairman, Quentin M. Geiman.

Since the officers and councilors approved another joint annual meeting with

the American Society of Parasitologists, an invitation was extended to that organization to meet with us in 1951. The American Society of Parasitologists accepted the invitation with the provision that the meeting be held in St. Louis, Chicago, or Indianapolis. Our officers and councilors approved of Chicago as a meeting place, with the National Malaria Society and the American Academy of Tropical Medicine in agreement. Each organization set up its own program committee with the understanding that the programs were to be coordinated and that a single combined program would be printed. The time for the meeting was set for November 15–17, with headquarters in the Congress Hotel. The Local Committee, made up of members of the organizations meeting conjointly, included Robert M. Lewert, Chairman; William Balamuth, Harold Elishewitz, and Viola Mae Young.

The call for papers was mailed to members June 15 with the deadline for the receipt of applications set at September 1. The number of applications for a place on the program was forty-four. Thirty-five papers were accepted for presentation at the scientific sessions, and nine applications were rejected. Five scientific exhibits were accepted for mounting, and one film was scheduled to be shown. A panel discussion entitled "Tropical Medicine in the Armed Forces" was organized.

The commercial exhibits mounted at this meeting were solicited and arranged for once again by Ernest Gamache, Executive Director of The American Foundation for Tropical Medicine. Twelve exhibits were accepted and shown, a higher number than was shown at any previous meeting. These exhibits proved to be of mutual benefit to members and exhibitors. In addition, they helped to defray the costs of the meeting.

Tropical Medicine and World Health—presented by Paul F. Russell.

The work of this committee was confined to a revision of a supplement to TROPICAL MEDICINE NEWS entitled "Health Hints for the Tropics." The revised manuscript is in the hands of the Secretary, and it will be forwarded to the Editor of TROPICAL MEDICINE NEWS for early publication.

Bailey K. Ashford Committee—The award was postponed until 1952.

Committee on Teaching of Tropical Medicine—submitted by Hamilton H. Anderson.

Members of the committee were consulted for data on new teaching programs in the United States. So far as could be determined, no outstanding new teaching techniques are being introduced. No effort was made to survey all schools as was attempted during World War II. Perhaps this should be done in the near future.

Amebiasis Committee—submitted by Marion M. Brooke, Chairman.

The Subcommittee on the Diagnosis of Amebiasis has as its objective the increase in the reliability of diagnosis (a) by determining the relative value of the morphological characteristics of *Endamoeba histolytica* and (b) by recommending acceptable laboratory procedures for the recovery and identification of the organisms. As one approach to the problem, a memorandum on the subject has been prepared for the purpose of obtaining additional information from other individuals interested in amebiasis. The memorandum consists of approximately 100

statements on clinical and laboratory aspects of diagnosis. The statements do not necessarily represent the opinions of the subcommittee, and some of the statements have been intentionally worded to be provocative.

The purpose of this memorandum is to obtain information which can be analyzed and interpreted by the subcommittee. It is not simply an attempt to obtain votes, pro and con, on various statements. It is realized that even perfect agreement by a group of individuals may not necessarily represent scientific truth. On the other hand, *divergence of opinion by individuals acquainted with the field may well indicate the need for more reliable fundamental information*. It is hoped that the analysis of the replies to the memoranda may assist in three ways: (a) by indicating a number of apparently reliable facts as based upon our present information, (b) by focusing attention upon the gaps in our knowledge, and (c) by stimulating needed research on the diagnosis of amebiasis.

At the 1950 annual meeting of the American Society of Tropical Medicine it was proposed that the memorandum be distributed to the membership of the Society. Toward the end of September, 1951, the memorandum was mailed to approximately 750 members residing in the United States. An accompanying note addressed to the members was designed to encourage replies only from individuals experienced in the field of amebiasis. As of November 13, the memorandum has been completed and returned by 50 members of the Society. Time has not permitted the analysis of these replies prior to the preparation of this report.

Last year, the memorandum was completed by 23 members of the Amebiasis Committee, and by 25 persons selected by members of the committee. The analysis of these 48 replies revealed that of 105 premises as stated in the memorandum the group indicated good agreement on 32%, fair agreement on 19%, poor agreement on 33%, and little or no agreement on 16%. There is not time to present further details of the analysis except to cite one point of particular interest: Of 25 statements that dealt with the identification of *Endamoeba histolytica* in feces and culture, the following in reference to conclusive proof of the organism was the only statement on which there was good agreement: "Cysts larger than 10 microns containing one to four Endamoeba-type nuclei and rod-shaped chromatoid bodies with smooth rounded ends (combination saline and iodine or permanent stained smears)."

Reports of representatives to other organizations:

National Research Council—submitted by Henry Meleney.

The meeting consisted of the presentation of the Annual Report of the Division of Medical Sciences and of various committees and representatives of affiliated societies. The President of the National Academy of Sciences, Dr. Detlev Bronk, addressed the meeting emphasizing that the Academy and the Council devoted considerable energy during the past five years to support of the Armed Forces and maintenance of liaison between these services and the civilian scientists of the country. He stated that the Council affords a means whereby civilian scientists can participate in the policy determinations of their government, making possible continued development of research and maintenance of the scientific spirit.

The Chairman of the National Research Council, Dr. Milton Winternitz, reviewed the functions of member societies. He stated that the number of member societies has increased from 12 to 21 since the Division of Medical Sciences was created in 1919. He pointed out that there was duplication in the fields represented by these societies, and also that there were other important fields which lacked representation. In the field of tropical medicine both the American Academy and the American Society are represented. The Nominating Committee was asked to make recommendations modifying the fundamental structure of the Division. It recommended that membership include representatives of each recognized field of medical research, that all societies in the United States sponsoring medical research according to recognized standards be allowed to submit nominations from which the Division would choose one or more representatives in each field. This recommendation was approved and submitted to the National Research Council for action. Dr. Winternitz also recommended that each Society choose as its representative an individual who is active in both the work of the Division and of his Society, and that representatives be chosen in consultation with the Division. He pointed out also that certain societies are now supporting fellowships, and suggested that each society support one or two fellowships.

The following reports from committees and societies are of interest to the American Society of Tropical Medicine:

1. *The Sub-Committee on Malaria.* This committee was appointed as an *ad hoc* committee at the request of the Army Medical Research and Development Board under the Chairmanship of Dr. Robert Loeb. It met on December 29, 1950, and made recommendations for research studies most urgently needed. This committee was then established as a permanent Sub-Committee on Malaria of the Committee on Medicine.

2. *Committee on Veterans' Medical Problems.* Among the follow-up studies of veterans one is being conducted on cases of schistosomiasis acquired by troops in action and prisoners of war of the Japanese under the direction of Dr. Harry Most. This project will be completed during the present fiscal year.

3. *Sub-Committee on Tropical Medicine.* Only 5 research projects were referred to this sub-committee during the past year. Action on these was taken by mail, and the sub-committee held no formal meeting. On June 16, 1951, Dr. Winternitz notified the members of this sub-committee that at the annual meeting of the Division of Medical Sciences the recommendation was approved to disband this sub-committee as of June 30, 1951, in line with reorganization of certain of the committees and sub-committees. In this connection it may be noted that the Division of Medical Sciences at the request of the Surgeon-General of the Navy has since established a Committee on Naval Medical Research to advise concerning the Navy's general plan of medical research. Dr. Harry Most, who was a member of the previous Sub-Committee on Tropical Medicine, has been appointed to this new Committee.

4. *Sub-Committee on Animal Reservoirs and Vectors of Disease.* Paul Russell was succeeded as Chairman of this sub-committee by C. W. Kruse of Johns Hopkins

University. A meeting was held in May, 1951, at which past activities were reviewed and a program of technical reports was adopted for the coming year.

5. *American Academy of Tropical Medicine.* This report, submitted by Clay G. Huff, consisted entirely of a report prepared by the Executive Director of the American Foundation for Tropical Medicine for the year 1950. The report gave a statement of the contributions made to the Foundation and to its Liberian Institute from 1945 through 1950 totaling $313,972. He stated that the Liberian Institute, which is now nearly completed, has also received a grant of $50,000 through Point IV Funds for operation for the current fiscal year under the supervision of the National Institutes of Health. Grants-in-aid during 1950 were made to support the Richard Pearson Strong Professorship of Tropical Medicine at the Harvard School of Public Health ($1,000) and to the American Society of Tropical Medicine ($1,209) for an exhibit on tropical diseases at the annual meetings of the American Medical Association and the American Society of Tropical Medicine. It was stated that broader efforts must be undertaken in fund raising in the future in order to furnish stability for a more effective program.

6. *American Society of Tropical Medicine.* This report emphasized the importance of the Society in promoting tropical medicine. It noted the plan to amalgamate the Society with the National Malaria Society. It also mentioned the activities of the Committee on amebiasis and the Committee on Tropical Medicine and World Health, and offered its services to the National Research Council and the Armed Forces in developing strategy for combating and controlling tropical infections through conference, research, and teaching.

American Association for the Advancement of Science—no report.

National Security Resources Board—no report.

Microbiological Certification Project—no report.

Amalgamation Committee—report presented by Quentin M. Geiman.

Since our Society and the National Malaria Society approved amalgamation of the two organizations, a Joint Amalgamation Committee was appointed by the presidents of the two organizations to draft a new constitution, to explore Journal policies, financial arrangements, and proper procedures to legalize the amalgamation. The National Malaria Society appointed Justin Andrews, S. W. Simmons, Martin D. Young, and Jack Henderson to serve on the Committee. The representatives of our Society included Paul Russell, Quentin M. Geiman, Donald L. Augustine, and William A. Sodeman. As a result of correspondence, this Joint Amalgamation Committee was divided into subcommittees to draft a new constitution, to make recommendations to govern publication policies, and to make recommendations regarding legal and financial aspects. The subcommittee to draft the constitution was composed of Paul Russell, Quentin Geiman, Samuel Simmons, and Martin Young; Journal policies, Justin Andrews and Donald Augustine; legal policies, Jack Henderson and William Sodeman. During the year G. Robert Coatney was appointed to replace Jack Henderson, and Hamilton Anderson was appointed as alternate to serve in the absence of William Sodeman.

Constitution Subcommittee: Two meetings were held in Boston to draft a new constitution and by-laws for circularization to members of the two organizations

for their approval. Considerable effort went into the preparation of this document. A semifinal copy as agreed upon by the Joint Amalgamation Committee was then submitted to the entire active membership of the two organizations with a request for a vote of approval. Of our total active membership of 863, approval was voted by 510 members, 8 members did not approve because of minor objections that could be met, and there was one negative vote. A number of minor changes were submitted by members, many of which were incorporated by the subcommittee before the final copy was submitted for approval by the Joint Amalgamation Committee on November 14 in Chicago. The final corrected copy of the constitution and by-laws will be published with the minutes of the amalgamation meeting (See Appendix.)

Subcommittee on Journal Policies and Publishing Agreements: This new journal shall include scientific articles of interest to those concerned with research, the practice of medicine, prevention of disease, and related public health and welfare considerations, particularly those related to tropical and arthropod-borne diseases.

In addition to this new journal, there will be printed and distributed a news bulletin to be known as "Tropical Medicine and Hygiene News." Both of these publications are to be prepared in the same editorial office with an undivided editorial responsibility.

This bulletin is to serve the same purpose as the present "Tropical Medicine News," suitably expanded to include the interests of public health workers in this field. It is recommended that the editor and his board determine the advisability of joint or separate mailing of the "Journal" and the "News."

With the combined publication resources of the American Society of Tropical Medicine and the National Malaria Society, it should be possible to publish a jounal of not less than 1200 pages containing material of interest to the clinician, epidemiologist, public health administrator, the person concerned with medical care, the scientist, engineer, public health educator, and the layman dealing with tropical affairs.

During the course of extensive discussions by members of the Joint Amalgamation Committee meeting on May 22, the name of the Journal was considered at length, the editorial policies and the make-up of the editorial board were explored thoroughly, and the publication policies and publishing agreement with the old or a new publisher were discussed in detail. The advisability of publishing an enlarged journal to accommodate the volume of scientific papers from the two parent societies was considered desirable. TROPICAL MEDICINE NEWS, being an unofficial publication of the Society without mention in the Constitution and By-laws, was approved for continuation as a result of vote by the active membership. The tabulation on the vote is as follows: to continue—285, to continue but include in Journal—209, to discontinue—13, no vote—3. The secretaries of the two parent societies and members of the committee were asked to explore the possibility of getting a new publisher for the Journal and News. The publisher of the AMERICAN JOURNAL OF TROPICAL MEDICINE, The Williams & Wilkins Company, of Baltimore, Maryland, were notified about the possibility of amalgamation and

were asked to submit terms of a new publishing agreement for the consideration of the committee. This latter subject led to a meeting of the committee with Mr. Passano, representing The Williams & Wilkins Company, on November 14 in Chicago. At the meeting, the committee expressed its dissatisfaction with the terms offered by the publisher, and the publisher was requested to modify the agreement so that it would be equitable to both the publisher and the owner of the Journal. The committee postponed a decision about the publisher and an agreement until the pertinent details could be further explored.

Subcommittee on Legal and Financial Aspects: Through the Secretary of the Society, this Subcommittee initiated its activities by obtaining advice concerning the legal procedures to dissolve the parent organizations and to form the amalgamated society. Since this Society was incorporated in Philadelphia under the laws of Pennsylvania on July 12, 1904, we were advised to retain counsel with a legal firm in Philadelphia. Since the National Malaria Society was not incorporated, such a procedure with that organization was unnecessary. The Society was further advised to pass a resolution at its annual meeting to dissolve itself and, upon legal dissolution, to assign its liabilities and assets to the new organization contingent upon similar action by the National Malaria Society. A group of individuals from the two parent societies was then advised to meet in Chicago to organize a new society composed of those members of the parent societies desiring to be affiliated with the new organization. If the new society was formed, our parent Society was to initiate proceedings for dissolution under the laws of Pennsylvania. When the dissolution becomes effective, the assets of the American Society of Tropical Medicine are to be transferred to the new organization. This procedure was approved by the Amalgamation Committee and provided the basis for the sequence of events that would lead to the legal amalgamation of the National Malaria Society with the American Society of Tropical Medicine and the formation of the American Society of Tropical Medicine and Hygiene.

Recommendations of Amalgamation Committee:

1. The Committee recommended that the constitution and by-laws as corrected be approved and recommended to the amalgamated society for adoption.

2. It was recommended that the publications of the society be the AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE and TROPICAL MEDICINE AND HYGIENE NEWS, headed by the editor and the editorial board.

3. The recommendation was made that a publisher be sought who would write an equitable agreement between publisher and owner of the publications.

4. It was further recommended that the following motion be presented at the annual business meeting for approval by the membership:

"I move (1) that as soon as practicable after the creation of the proposed American Society of Tropical Medicine and Hygiene, the Secretary-Treasurer of the American Society of Tropical Medicine, Inc., be empowered to petition the State of Pennsylvania to dissolve this corporation in accordance with its laws, with the understanding and agreement that all assets, liabilities, and membership of the dissolved corporation be transferred as soon as possible to the American Society of Tropical Medicine and Hygiene and (2) that the Secretary-Treasurer

of the American Society of Tropical Medicine, Inc., be authorized to employ and compensate legal counsel for this purpose."

The report of the Joint Amalgamation Committee was accepted as presented, and the presentation of the motion was approved by the officers and councilors. The motion to be presented at the annual business meeting was approved.

Nominations for 1951 Officers: The council nominated the following slate of officers for consideration at the annual business meeting of the Society. Even though amalgamation of this organization with the National Malaria Society was scheduled to take place, a full roster of officers and councilors is needed to officially conduct the business of the organization until it can be dissolved legally. For president-elect, Harry Most. For vice-president, Martin D. Young. For councilors, W. Clark Cooper and Clyde Swartzwelder.

Committee Appointments: In view of the scheduled amalgamation proceedings with the National Malaria Society and the dissolution of this Society, all committees appointed for 1951 are to remain in office.

New Business: Since it is extremely advantageous to decide on a meeting place for the 1952 annual meeting, a motion was presented and passed that the annual meeting be held in Galveston, Texas, upon the invitation of the University of Texas Medical Branch.

THE MINUTES OF THE ANNUAL BUSINESS MEETING

The annual business meeting of the Society was held on November 16 at 8:30 a.m. in the Gold Room of the Congress Hotel, Chicago, Illinois. The minutes of the previous meeting were accepted as published in the Journal, May, 1951. The Secretary-Treasurer's report was approved as read. Dr. Lewis Hackett, Editor of the Journal, presented the Journal report. The report was accepted as read, with the President commending the Editor on behalf of the Society for his outstanding achievement during the year. The report of the Editor of TROPICAL MEDICINE NEWS was accepted as read. The auditors, George M. Saunders and G. Robert Coatney, who were appointed by the President to examine the Treasurer's books reported the account to be in order.

At this time, the President appointed Herbert G. Johnstone and George M. Saunders as sergeants-at-arms to assemble a quorum of members registered at the annual meeting and to act as tellers of votes on the recommendations submitted for action.

The following recommendations passed by the Council were presented to the Society for action:

1. Since the Secretary-Treasurer postponed the purchase of a new Addressograph machine last year because of the pending amalgamation, permission to purchase a new machine during the coming year is recommended.

2. It is further recommended that the Secretary-Treasurer be allotted a sum not to exceed $110 per month for secretarial assistance during the coming year.

3. It is further recommended that the Secretary-Treasurer be authorized to engage a certified public accountant to close out the accounts of the Society.

4. It is finally recommended that the Editor of the Journal submit a bill for

reimbursement to the Treasurer for added expenses of his office incurred during the past year and that a satisfactory budget be worked out for the coming year.

Approval of these recommendations was voted.

Condensed reports of committees and representatives to other organizations presented previously to the officers and councilors were read. To inform the members about the amalgamation proceedings, the Secretary gave a more detailed account of the work of the Joint Amalgamation Committee. Approval of these reports was voted.

The following officers and councilors were elected unanimously to carry on the business of the Society during the interim prior to dissolution:

President-Elect—Harry Most Vice-President—Martin D. Young
Councilors—W. Clark Cooper and Clyde Swartzwelder

The President announced that the interests of the Society would best be served by keeping committees appointed for 1951 in office until the Society is dissolved. A motion was made and carried for the existing committees to remain in office pending dissolution.

New Business: Tom F. Whayne, representing the Resolutions Committee, recommended that the Secretary be instructed to transmit letters of appreciation to the following:

Families of deceased active and honorary members,
Participants in the Panel Discussion,
Host organizations—The University of Chicago, Roosevelt College, The University of Illinois Medical School, Northwestern University, The Chicago Medical School,
Dr. Robert M. Lewert, Chairman of the Local Committee, and each member of his committee for their fine work,
Mrs. R. M. Lewert, Chairman of the Committee for Ladies' Entertainment and each member of her committee,
Sponsors of Hospitality Sessions—Abbott Laboratories, Julius Hyman Co. and Shell Chemical Corp., and Parke, Davis and Co.,
Mr. Ernest Gamache of the A.F.T.M. for organizing the commercial exhibits,
Sponsor of ladies' entertainment—Velsicol Corporation,
Manager of the Congress Hotel,
Dr. Lewis W. Hackett, Editor of the Journal, for his stimulating report and outstanding contribution to the welfare of the Society,
Officers and councilors for their services,
The National Malaria Society for its cooperation in achieving amalgamation.

The recommendation was voted.

A motion to make Mark F. Boyd, past editor of the Journal, an emeritus member of the Society was presented by M. E. Barnes and voted unanimously.

The following motion concerning the dissolution of the American Society of Tropical Medicine was presented by Justin Andrews:

"I move (1) that as soon as practicable after the creation of the proposed American Society of Tropical Medicine and Hygiene, the Secretary-Treasurer of the American Society

of Tropical Medicine, Inc., be empowered to petition the State of Pennsylvania to dissolve this corporation in accordance with its laws, with the understanding and agreement that all assets, liabilities, and membership of the dissolved corporation be transferred as soon as possible to the American Society of Tropical Medicine and Hygiene and (2) that the Secretary-Treasurer of the American Society of Tropical Medicine, Inc., be authorized to employ and compensate legal counsel for this purpose.

"At the same time the above motion was presented, 181 members of the American Society of Tropical Medicine were in attendance at the annual meeting, of which 91 members constitute a quorum to transact business.

"The above motion was seconded and voted as follows:

"Total vote cast for above motion:

Approved—105
Not approved— 0

"Verified and signed by tellers,
/s/ Herbert G. Johnstone
/s/ G. M. Saunders."

Finally, the President announced that the officers and councilors accepted the invitation of the University of Texas Medical Branch to hold the next annual meeting in Galveston, Texas. The dates for the meeting will be November 13–15, 1952.

Respectfully submitted for publication,
Quentin M. Geiman
Secretary-Treasurer

SCIENTIFIC SESSIONS

The first scientific session of the Society consisted of a panel discussion entitled "Tropical Medicine in the Armed Forces." The panel discussion was held in the Gold Room of the Congress Hotel at 2 p.m., November 15.

PANEL DISCUSSION

TROPICAL MEDICINE IN THE ARMED FORCES

1. Tropical Medicine in the Armed Forces—Introduction. (20 min.) Colonel William S. Stone, Commandant, Army Medical Service Graduate School, Army Medical Center, Washington, D. C.
2. Schistosomiasis and Molluscacides. (20 min.) Dr. Donald B. McMullen, University of Oklahoma Medical School, Oklahoma City, Oklahoma.
3. Dermatologic Disability Induced by Topical Medication. (20 min.) Dr. Donald M. Pillsbury, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
4. Leptospirosis in North America. (20 min.) Lt. Colonel Robert H. Yager and Major William S. Gochenour, Jr., Army Medical Service Graduate School, Army Medical Center, Washington, D. C.
5. Filariasis in American Samoa. II. Evidence of Transmission Outside of Villages. (20 min.) Lt. Leo A. Jachowski, Jr., and Dr. Gilbert F. Otto, Naval Medical Research Institute, Bethesda, Md., and School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
6. Dysentery in Korea. (20 min.) Dr. Albert V. Hardy, Bureau of Laboratories, Florida State Board of Health, Jacksonville, Florida.

The second scientific session of the Society was held in the Gold Room of the Congress Hotel at 9:30 a.m., November 16, following the business meeting of the Society.

1. Hetrazan in Experimental Trichinosis. (8 min.) Thomas B. Magath and John H. Thompson, Jr., Mayo Clinic, Rochester, Minnesota.
2. Studies on Hookworm Disease in Puerto Rico—Preliminary Report. (8 min.) R. Rodriguez-Molina and Jose Oliver-Gonzalez, Veterans' Administration Center, San Patricio Hospital, San Juan, P. R., and University of Puerto Rico, School of Medicine, San Juan, P. R.
3. The Incidence and Clinical Aspects of Schistosomiasis japonica in American Military Personnel Formerly Interned by the Japanese in the Davao Penal Colony. (12 min.) Harry Most, Lt. Col. Robert Traub, Major Lyman P. Frick, Ethyl Ringer, and Ethel Ballard, N. Y. U. College of Medicine, New York, and Army Medical Service Graduate School, Washington, D. C.
4. Schistosomiasis in the Dominican Republic. (8 min.) Louis J. Olivier, Charles M. Vaughn and James R. Hendricks, National Institutes of Health, Bethesda, Md., The Bowman Gray School of Medicine, Winston-Salem, N. C., and School of Public Health, University of North Carolina, Chapel Hill, N. C.
5. Unusual findings of Filaria Infections of Man. (8 min.) Ernest Carroll Faust, Moises Agosin, Alberto Garcia-Laverde, W. Y. Sayad, and V. M. Johnson, Tulane University, New Orleans, La., Universidad de Chile, Santiago, South America, Universidad Nacional, Caracas, and Good Samaritan Hospital, West Palm Beach, Fla.
6. Chemotherapy of Experimental *Schistosomatium douthitti* Infections in Mice. (8 min.) Irving G. Kagan and Chang-Ling Lee, The University of Chicago, Chicago, Illinois. (Introduced by W. H. Taliaferro.)
7. The Effects of Orally Administered Terramycin on Certain Human Helminthiases. (8 min.) Elmer H. Loughlin, Irving Rappaport, Helen S. Wells, William G. Mullin and Aurele A. Joseph, with the assistance of Hodelin Rene, The Institute of Inter-American Affairs, Port-au-Prince, Haiti, and the Long Island College, Brooklyn, New York.
8. Fish Poisoning—a Neglected Military-Public Health Problem of the Tropical Pacific. (10 min.) Bruce W. Halstead, College of Medical Evangelists, Loma Linda, California.
9. Some Experiments on the Utilization of Carbohydrates by *Endamoeba histolytica*. (12 min.) Charles W. Rees, National Institutes of Health, Bethesda, Md.
10. Presence of a Growth Factor for *Endamoeba histolytica* in the Killed Cells of Associated Microorganisms. (8 min.) Jon L. Karlsson, School of Medicine, University of California, San Francisco, Calif. (Introduced by H. H. Anderson.)
11. The Experimental Transmission of *Endamoeba coli* and *Giardia lamblia*. (8 min.) Robert C. Rendtorff, Laboratory of Tropical Diseases, National Institutes of Health, Seagoville, Texas.
12. Acquired Immunity to Amebic Infection in Dogs. (8 min.) J. C. Swartzwelder and W. H. Avant, Louisiana State University School of Medicine, New Orleans, La.
13. Comparison of the Effectiveness of Proctoscopic and Stool Examination for the Detection of Amebiasis. (8 min.) Marion Hood, William A. Sodeman, and W. R. Akenhead, Louisiana State University School of Medicine, New Orleans, La., Tulane University School of Medicine, New Orleans, La., and L. S. U. School of Medicine, New Orleans, La.
14. Chemotherapy of *Endamoeba histolytica* Infections in the Guinea Pig. (8 min.) D. Jane Taylor and Joseph Greenberg, National Institutes of Health, Bethesda, Md.
15. Fumagillin in Amebiasis. (8 min.) H. H. Anderson, A. K. Hrenoff, J. Van D. Anderson, M. Nakamura, and A. N. Contopoulos, University of California, San Francisco, California, and American University of Beirut, Lebanon.
16. Experimental Studies on a New Type of Antiamebic Agent, (5-chloro-7-(diethyl aminomethyl)-8-quinolinol, dihydrochloride). (8 min.) Paul E. Thompson, J. W. Reinertson, Mary C. Dunn, and Anita Bayles, Research Laboratories, Parke, Davis and Company, Detroit, Michigan.

To be Read if Time Permits

17. Loa Loa Infection: Clinical Observations on the Disease and its Treatment with Hetrazan. (8 min.) Howard B. Shookhoff and Max M. Sterman, and Kermit G.

Dwork, Health Department, City of New York, and Columbia University School of Medicine, New York, N. Y.

18. A Search for Liver Damage in Anti-Amoebic Treatment with Diodoquin, Vioform and Carbarsone. (8 min.) Alva A. Knight and Donald W. Tarun, University of Illinois and Presbyterian Hospital, Chicago, Illinois.

The third scientific session was held in the Gold Room of the Congress Hotel on November 17. The following program was presented at 9:30 a.m. at the close of the amalgamation meeting.

1. The Sixteenth Annual Charles Franklin Craig Lecture: Charles Franklin Craig—Fifty Years of work and Service in Tropical Medicine. (30 min.) Charles C. Bass, School of Medicine, Tulane University, New Orleans, Louisiana.
2. Present Known Distribution of Q Fever in Texas. (8 min.) R. B. Eads, C. W. Johnson, K. S. Young and J. V. Irons, Texas State Department of Health, Austin, Texas, and Communicable Disease Center, Atlanta, Georgia.
3. Neutralizing Antibodies Against Certain Recently Isolated Viruses in the Sera of Human Beings Residing in East Africa. (8 min.) Kenneth C. Smithburn, The Rockefeller Foundation, New York.
4. *Anopheles earlei* Vargas, 1943, in Montana: Identity and Adaptation to Laboratory Conditions. (8 min.) Lloyd E. Rozeboom, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md.
5. Experimental Transmission of Tularemia by the Tropical Rat Mite. (8 min.) Cluff E. Hopla, University of Kansas and University of Oklahoma, Norman, Oklahoma.
6. Laboratory Studies on Warfarin and Related Rodenticides. (12 min.) Wayland J. Hayes, Jr., and Thomas B. Gaines, U. S. Public Health Service, Communicable Disease Center, Technical Development Services, Savannah, Georgia.
7. Histopathological Studies of Reactions to the Bites of Various Arthropods. (8 min.) Leon Goldman, Evelyn Rockwell and Daniel Richfield, College of Medicine, University of Cincinnati, Cincinnati, Ohio.
8. Entomological Studies on the Onchocerciasis Complex. IX. Insecticidal Control of Simulium. Area Treatment of Breeding Streams with DDT. (8 min.) H. Elishewitz and Eloy A. Barreda, The Chicago Medical School, Chicago, Illinois, and Institute of Inter-American Affairs.
9. Endemic Treponematosis (Balash or Bejel) in Saudi Arabia. (8 min.) H. T. Chaglassian, N. Bustani and Hamilton H. Anderson, American University, Beirut, Lebanon and University of California, San Francisco, California.
10. Further Observations on *Trypanosoma ariarii* of Colombia, South America. (8 min.) Hernando Groot, Sanitago Renjifo and Cesar Uribe, Army Laboratory, Bogota, Colombia, South America.
11. Diasone and Sulphetrone as Therapeutic Agents in Experimental Toxoplasmosis. (8 min.) Joy Barnes Cross, University of Texas Medical Branch, Austin, Texas.

To be Read if Time Permits

12. A Study of Intestinal Parasitism in New Hope Community, Jackson County, Tennessee. (8 min.) Don E. Eyles, Frances E. Jones, and C. S. Smith, Laboratory of Tropical Diseases, U.S.P.H.S., Memphis, Tennessee.
13. The Changing Picture of Salmonellosis. (8 min.) Oscar Felsenfeld, Franz J. Rutten and Robert Devignat, Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Illinois.
14. New Developments in Bismuth Chemotherapy. (8 min.) Ernst A. H. Friedheim, New York, N. Y.
15. Infectivity to Mosquitoes of Early Primary Parasitemias of *Plasmodium vivax* (Chesson strain). (8 min.) Geoffrey M. Jeffrey, National Microbiological Institute, U.S.P.H.S., Milledgeville, Georgia.

The fourth scientific session of the Society was held jointly with the National Malaria Society and the American Society of Parasitologists in the Casino Room of the Congress Hotel on November 17. The meeting was called to order at 2 p.m.

1. Research Needs in Tropical Medicine. (5 min.) W. W. Frye, Chairman, Tropical Medicine Study Section, Division of Research Grants and Fellowships, U.S.P.H.S.
2. The ECA Malaria Control Program in Vietnam—A Progress Report. (10 min.) H. D. Pratt, Communicable Disease Center, U. S. Public Health Service, Atlanta, Ga.
3. Sardinia, the Site of Anti-Anopheline Operations, 1946–1950. (12 min.) Thomas H. G. Aitken, The Rockefeller Foundation, New York, N. Y.
4. The Treatment of *Plasmodium falciparum* Malaria with a New Single Dose Antimalarial. (10 min.) Elmer H. Loughlin, Justus B. Rice, Helen S. Wells, Irving Rappaport, and Aurele A. Joseph, with assistance of Hodelin Rene, Institute of Inter-American Affairs, Port-au-Prince, Haiti, and Washington, D. C.
5. Epidemiological Appraisal of Malaria in the United States during 1951. (10 min.) Sarah F. Welch and Griffith E. Quinby, Communicable Disease Center, U. S. Public Health Service, Atlanta, Ga.
6. Malaria in Korean Casualties. (8 min.) Robert E. Blount, Col. Brooke Army Hospital, Fort Sam Houston, Texas.
7. Studies on Korean Malaria in Returning Troops. (8 min.) Martin D. Young and Robert W. Burgess, Laboratory of Tropical Diseases, National Institutes of Health, Columbia, South Carolina.
8. Entomological Studies on the Onchocerciasis Complex. VII. The Biting and Feeding Characteristics of Simulium. (10 min.) H. Elishewitz, The Chicago Medical School, Chicago, Illinois.
9. Forest Canopy Mosquitoes in Western Panama and Costa Rica in Relation to Sylvan Yellow Fever. (12 min.) Harold Trapido, Pedro Galindo and Stanley J. Carpenter, Gorgas Memorial Laboratory and U. S. Army.
10. Comparative Susceptibility of Common Laboratory Animals to Experimental Infection with *Schistosoma haematobium*. (10 min.) Donald V. Moore and Henry E. Meleney, New York University, College of Medicine, New York, N. Y.
11. Operation Santobrite—A Schistosome Snail Eradication Program in Japan. (12 min.) G. W. Hunter III, L. S. Ritchie, R. E. Freytag, C. Pan, and D. E. Potts, 406th Medical General Laboratory and National Institute of Health and Institute of Public Health and Welfare, Tokyo, Japan.
12. Some Effects of Cultural Associates on the Infectivity of a Strain of *Endamoeba histolytica*. (12 min.) George W. Luttermoser and Bruce P. Phillips, National Microbiological Institute, Bethesda, Md.

SCIENTIFIC EXHIBITS

The following scientific exhibits were displayed in the Pine Room of the Congress Hotel:

"Stool Examination for Salmonellae, Shigellae and Cholera Vibrios." Oscar Felsenfeld, Robert W. Edwards and Tama Yoshimara, Hektoen Institute for Medical Research, Cook County Hospital, Chicago, Ill.

"Penetration Studies (*Schistosoma mansoni*): I. Photomicrographs of Histological Sections of Cercariae in Mouse Tail Skin, II. Demonstration of an Enzymatic Factor in Cercariae by the Streptococcal Decapsulation Test, III. Some Conditions Which Influence Cercarial Penetration." M. A. Stirewalt, A. S. Evans, M. H. Powell, Carlos Schultz and J. R. Hackey, Naval Medical Research Institute, Bethesda, Md.

Exhibit, Armed Forces Institute of Pathology, Washington, D. C.

"A New Method of Rearing *Triatoma* Bugs and Infecting Them With *Trypanosoma cruzi*." Harry D. Pratt, Communicable Disease Center, U. S. Public Health Service, Atlanta, Ga.

"Yaws Control with Antibiotics." Elmer H. Loughlin, Aurele A. Joseph, and Irving Rappaport, with the assistance of Hodelin Rene, The Institute of Inter-American Affairs, Port-au-Prince, Haiti.

BUSINESS MEETING TO FORM THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE*

The business meeting to form the American Society of Tropical Medicine and Hygiene was held in room 785 of Roosevelt College, Chicago, Illinois, at 8:35 o'clock, Saturday morning, November 17, 1951, with Paul Russell presiding as temporary chairman. Chairman Russell called the meeting to order and moved that Justin Andrews be chosen as chairman of the meeting. This motion was seconded and voted favorably. Justin Andrews assumed the chair and called for the election of a temporary secretary. Quentin M. Geiman was nominated for secretary *pro tem*. The motion was seconded and unanimously carried. Chairman Andrews then called on Paul Russell to state the purpose of the meeting. Dr. Russell responded as follows: "Be it resolved that the new society is hereby formed by the assembled group, this society to be known as the American Society of Tropical Medicine and Hygiene, and that this resolution carries acceptance of the Constitution and By-laws (See Appendix) previously circularized to, and approved by, the majority of individuals here assembled, and that the Society's secretary-treasurer to be elected is hereby given the authority to proceed by due process of law to incorporate the Society.

"The purpose of this Society shall be the advancement of tropical medicine and hygiene, including medicine, nursing, engineering, entomology, parasitology, and allied specialties in this field."

A motion to accept the resolution was requested by the Chair. The motion was seconded and unanimously accepted. Since the Joint Amalgamation Committee of the two parent societies was empowered to submit a slate of officers and councilors for election, the Secretary was asked to present the slate as nominated by the Co nmittee. The Secretary responded as follows: "The Joint Amalgamation Committee met to consider the problems of amalgamation and in due course considered the slate of officers for this new society. Since the parent organizations had somewhat different purposes, great pains have been taken to name a slate that represented the different make-up of the two parent societies. The following slate was named and approved by the Joint Amalgamation Committee:

President: Martin D. Young

President-Elect: William A. Sodeman

Vice-President: H. W. Van Hovenberg

"No secretary-treasurer or editor of the Journal were named because provision was made under the Constitution and By-laws for their appointment and election by the officers and councilors. The councilors, however, that were nominated are: George H. Bradley and Harry Most for one year; Justin M. Andrews and Donald L. Augustine for two years; Tom F. Whayne and Herbert G. Johnstone for three years; and Edwin H. Lennette and G. Robert Coatney for four years."

The Chair requested the pleasure of the group about considering the slate as a

* A verbatim account of this meeting is on file in the archives of the new Society.

whole or member by member. It was moved and seconded that the slate of officers as read by the Secretary be elected. The nominations were closed, and the slate of officers was unanimously elected. J. C. Swartzwelder and Marshall Hertig were appointed as tellers to count and record the votes. 121 affirmative votes were recorded. The first president of the new Society, Martin D. Young, was congratulated by Chairman Andrews, after which President Young assumed the chair. Chairman Young proceeded to call on the representatives of the parent organizations to read the resolutions which led to the creation of the new society. Samuel W. Simmons presented the resolution which had been presented to the National Malaria Society on November 15 by Paul Russell: "It is moved that, since the objective of the National Malaria Society has been attained through the elimination of malaria as a major public health problem in the United States, as soon as it is conveniently possible the National Malaria Society, upon the formation of the American Society of Tropical Medicine and Hygiene, shall transfer its total assets, liabilities, and membership to the new society, and by this action shall dissolve itself."

Justin Andrews was then called upon to read the following resolution passed by the American Society of Tropical Medicine: "I move (1) that as soon as practicable after the creation of the proposed American Society of Tropical Medicine and Hygiene, the Secretary-Treasurer of the American Society of Tropical Medicine, Inc., be empowered to petition the State of Pennsylvania to dissolve this corporation in accordance with its laws, with the understanding and agreement that all assets, liabilities, and membership of the dissolved corporation be transferred as soon as possible to the American Society of Tropical Medicine and Hygiene and (2) that the Secretary-Treasurer of the American Society of Tropical Medicine, Inc., be authorized to employ and compensate legal counsel for this purpose."

President Young then announced that the first annual meeting of the new Society will be held in Galveston, Texas. He requested that the resolution to form the American Society of Tropical Medicine and Hygiene be signed by all individuals attending this meeting since those signatures are required for the incorporation of the new organization. President Young also requested a meeting of the new officers and councilors in an adjacent room immediately following adjournment. A motion to adjourn was then carried.

Respectfully submitted for publication,
Quentin M. Geiman
Secretary, *pro tem.*

MINUTES OF THE COUNCIL MEETING AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE*

The meeting of the officers and councilors of the American Society of Tropical Medicine and Hygiene was held at Roosevelt College, Chicago, Illinois, at 9:10 a.m., November 17, 1951. The meeting was called to order with President Martin D. Young presiding.

* A verbatim account of this meeting is on file in the archives of the new Society.

The first order of business was the election of a secretary-treasurer for three years. Quentin M. Geiman was nominated and unanimously elected.

The next order of business was the appointment of an Editor of the Society's publications. Lewis W. Hackett was nominated and elected by a unanimous vote.

Appointment of Committees: Before proceeding with this important item, the Secretary raised a question about the number and names of committees needed by the new Society, stating that the council should be open-minded in creating any committees over and above those that are needed annually to help carry on the business of the Society and to make awards. The Constitution and By-laws were consulted before proceeding. The following committees were appointed: Nominating Committee: H. H. Anderson, Chairman; W. H. Wright; N. H. Rector. Membership Committee: G. F. Otto, Chairman; H. Most; W. D. Gingrich. Honorary Membership Committee: P. F. Russell, Chairman; M. Hertig; R. E. Dyer. The Program Committee, the committees on awards, and various other committees were to be appointed at a later date.*

New Business: The Secretary-Treasurer was empowered by a motion to transact the business of the Society, to pay the necessary bills and to pay up to $110 per month for secretarial help. It was further understood that a certified public accountant would be retained to set up the books of the Society and to audit the account before the annual meeting. The request of the Secretary-Treasurer to be bonded for the amount of $15,000 was moved and voted.

A motion was made and carried to grant to the Editor the necessary expenses for running his office.

Agreement was reached to set up a Committee on Teaching and Research in Tropical Medicine and Hygiene, to appoint one representative to the National Research Council, to appoint two representatives to the American Association for the Advancement of Science, and to create a Policy Committee to help guide the officers and councilors during the initial years of this new organization.

Finally, agreement was reached that a two-thirds vote of the officers and councilors would be needed for approval of any items of business requiring a decision during the year. The meeting was adjourned.

Respectfully submitted for publication,
Quentin M. Geiman
Secretary-Treasurer

* *Program Committee:* Q. M. Geiman, Chairman; S. W. Simmons; A. J. Warren; and L. W. Hackett, ex-officio.

Policy Committee: J. M. Andrews, Chairman; C. B. Philip; J. C. Snyder; J. F. Kessel.

Teaching and Research in Tropical Medicine and Hygiene: W. W. Frye, Chairman; H. W. Brown; E. H. Hinman.

C. F. Craig Lecture Committee: H. E. Meleney, Chairman; A. C. Chandler; L. L. Williams.

Bailey K. Ashford Award Committee: L. E. Rozeboom, Chairman, 1952; J. F. Kessel, 1954; W. A. Sodeman, 1956.

Walter Reed Medal Committee: (Three past-presidents) J. S. Simmons, Chairman, 1953; N. H. Topping, 1955; N. P. Hudson, 1957.

Representative to the National Research Council: H. Most (two years).

Microbiological Certification Project: W. H. Taliaferro (temporary); W. H. Wright.

Representatives to the American Association for the Advancement of Science: E. C. Faust; Q. M. Geiman.

APPENDIX

CONSTITUTION AND BY-LAWS OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

Organized November 17, 1951, Chicago, Illinois

Incorporated Under the Laws of the State of Delaware, January 21, 1952

CONSTITUTION

ARTICLE I

NAME, ORIGIN, AND PURPOSE

Section 1

This society shall be called the "American Society of Tropical Medicine and Hygiene."

Section 2

The Society shall be incorporated under the laws of the State of Delaware and shall be formed by the amalgamation of the American Society of Tropical Medicine, founded in 1903, and the National Malaria Society, founded in 1916.

Section 3

The purpose of this Society shall be the advancement of tropical medicine and hygiene, including medicine, nursing, engineering, entomology, parasitology, and allied specialties in this field.

ARTICLE II

MEMBERSHIP

Section 1

All members in good standing as of the date of amalgamation of the American Society of Tropical Medicine and of the National Malaria Society become charter members of the American Society of Tropical Medicine and Hygiene upon payment of the annual dues.

Section 2

There shall be four classes of members: Active, Honorary, Life, and Emeritus.

Section 3

Individuals regardless of race or nationality interested in any phase of tropical medicine and hygiene, including all arthropod-borne diseases, medical care, and human welfare, may be proposed for Active Membership and may be elected as provided by the By-laws of the Society.

Section 4

Any individual not an American citizen who has made eminent contributions to some phase of tropical medicine or hygiene shall be eligible for Honorary Membership by election as provided in the By-laws of the Society. Such Honorary Members shall not vote or pay dues, but shall receive the official publications of the Society.

Section 5

Any Active Member may become a Life Member by payment of $200 in one sum, by which he becomes exempt from the payment of any future dues assessable during his lifetime. Each Life Member shall continue to exercise all rights of an Active Member and to receive the official publications of the Society during his lifetime.

Section 6

The Council may transfer Active Members in good standing to Emeritus Membership if the individual concerned has so requested. Such Emeritus Members shall not vote, pay dues, or receive the official publications of the Society. Eligibility for Emeritus Membership consists of retirement from active work, foreign duty, or service in the armed forces. Emeritus Members may be reinstated as Active Members upon request.

ARTICLE III

Officers

Section 1

The Officers of the Society shall be a President, a President-Elect, a Vice-President, a Secretary-Treasurer, an Editor, and eight Councilors. Any member in good standing is eligible for election to office. The Officers shall constitute the Council in which the government of the Society shall be vested.

The President-Elect and the Vice-President shall be elected by the Society for a term of one year or until their successors are elected. The President-Elect at the end of his term shall become the President of the Society for the ensuing year.

The Secretary-Treasurer and Editor of Society publications shall be elected by the Council for a term of three years.

Two Councilors shall be elected by the Society at each annual meeting to serve for four years.

Terms of office in the Society shall begin at the close of the annual meeting at which the officers were elected.

If the Presidency becomes vacant during an unexpired term, the President-Elect shall become President and continue in office during the term for which he was elected. The unexpired term in the office of President-Elect shall be filled by the Vice-President until the next annual meeting. The President shall appoint one of the senior Councilors to fill a vacancy in the office of the Vice-President until the next election. Vacancies among the Councilors occurring during the year may be filled by appointment by the President to serve until the next annual meeting.

Section 2

A Nominating Committee of three members representing the medical, sanitary engineering, and biologic interests of the Society shall be appointed by the Council at each annual meeting. Six months before the next annual meeting, the Nominating Committee shall submit to the Secretary the names of two or more nominees for each officer to be elected.

The Secretary shall send ballots to each member with the request that his vote be returned at least one month prior to the annual meeting. The President shall appoint a committee to count the ballots at the annual meeting so that the results can be announced in the business meeting.

ARTICLE IV

Publications of the Society

Section 1

The Society shall publish two bimonthly publications, the American Journal of Tropical Medicine and Hygiene, and Tropical Medicine and Hygiene News.

ARTICLE V

Amendment of Constitution

Amendments to this Constitution may be adopted at the annual meeting of the Society upon approval by the Officers and Councilors and upon a two-third affirmative vote of ballots cast, and subject to the following conditions:

a. The proposed amendment must be in writing and signed by at least five members of the Society.

b. The signed proposed amendment must be in the hands of the Secretary at least six months before the annual meeting of the Society.

c. The Secretary shall mail copies of the proposed amendment to all members of the Society for their vote at least one month before the annual meeting.

d. Ballots by mail shall be counted and the results announced at the annual meeting.

BY-LAWS

ARTICLE I

The duties of the President shall be those regularly devolving on the chief executive officer. He shall preside at meetings of the Council and the Society, appoint committees as provided in the By-laws, and he shall deliver an address at the annual meeting.

ARTICLE II

The President-Elect shall succeed the President at the annual meeting except as noted in Article III of the Constitution.

ARTICLE III

The Vice-President shall preside at meetings in the absence of the President, and he shall assist the Program Committee in duties involved with the annual meeting of the Society.

ARTICLE IV

The Secretary-Treasurer shall be the custodian of all records of the Society. He shall handle the business and finances of the Society and shall submit an annual financial report approved by a certified public accountant. He shall be responsible for the arrangement of meetings and appointment of local committees. He shall maintain the financial account of Society publications. He shall attend and record the minutes of meetings and be reimbursed from the funds of the Society for expenses incurred. He shall be bonded for a sum to be determined annually by the Council.

ARTICLE V

The Council shall manage the affairs and direct the business of the Society. It shall initiate, coordinate, and terminate the work of the committees. It shall elect the Secretary-Treasurer and Editor of publications. It shall have the power to approve the expenditure of funds and establish an annual budget for the publications of the Society.

ARTICLE VI

The Editor of Society publications with the aid of the Editorial Board is responsible for the form and content of publications of the Society. The Editorial Board shall be composed of eight members to be selected and changed by the Editor subject to approval by the Council.

ARTICLE VII

Nominations for membership shall be made on the official application blank and be endorsed by two members. To be elected, the applicant must be approved by the Membership Committee.

ARTICLE VIII

Honorary Members shall be nominated by the Honorary Membership Committee, approved by unanimous vote of the Council and elected by the Society.

ARTICLE IX

The Treasurer shall open a special account for the reception of payments of Life Memberships, of which the income only may be applied to the operating expenses of the Society,

and upon the death of any Life Member the amount of his membership fee shall be transferred to and become part of an endowment fund of the Society, the income of which may be expended in accordance with such regulations as the Society shall adopt.

ARTICLE X

The annual dues of the Society shall be eight dollars which shall become due and payable the first day of January of each year. Active Members in good standing shall receive the annual volume of the Journal and published issues of the News. Any member in arrears of dues for three years shall be dropped from membership.

ARTICLE XI

Unless otherwise ordered by the Council, there shall be an annual meeting of the Society consisting of business and scientific sessions. The time and place for the annual meeting is to be determined by the Secretary-Treasurer with the approval of the Council. In the conduct of the business sessions, Robert's Rules of Order shall be the guide to the parliamentary procedure. The minutes of the annual meeting shall be published in the Journal.

ARTICLE XII

The President shall appoint annually a Membership Committee, a Committee on The Charles Franklin Craig Lecture, and an Honorary Membership Committee, each composed of three members. The President shall appoint two members of the Society to serve on the Program Committee with the Secretary-Treasurer as Chairman, and the Editor as an ex-officio member.

The Council shall appoint one Past-President every second year for a term of six years to the Committee on Award of the Walter Reed Medal composed of three Past-Presidents selected from the parent Societies or this Society; it shall appoint a member of the Society every second year for a term of six years to the Committee on the Bailey K. Ashford award; it shall appoint every third year the Committee on the Joseph Augustin LaPrince Award to consist of the President of the Society as Chairman, the immediate Past-President and the President-Elect; and it is responsible for creating, appointing, and discontinuing other committees required to transact the business of the Society.

ARTICLE XIII

The By-laws may be amended at the annual meeting by a two-third vote of members present and voting, provided such amendments have been sent to the Secretary-Treasurer for circularization to the Council at least three months before the meeting.

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgment must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

International Health Organizations and Their Work. By Neville M. Goodman, M.D., F.R.C.P., D.P.H., formerly on the Health Committee of the League of Nations; British Delegate to the International Office, Paris; Director of Health for UNRRA in Europe; Director of Field Services, Interim Commission, W.H.O.; now Assistant Director-General (Acting), W.H.O. and Lecturer on International Health, London School of Hygiene and Tropical Medicine. 327 pp., 53 illustrations. Cloth. Philadelphia-New York, The Blakiston Company, 1952. Price $6.50.

Médecine Tropicale. By Marcel Vaucel, Inspecteur général des Instituts Pasteur d'Outre-Mer, Ancien directeur du Service de Santé au Ministère de la France d'Outre-Mer, avec 14 collaborateurs. Dans la Collection Médico-Chirurgicale a Revision Annuelle (Directeur: Pasteur Vallery-Radot). Deux volumes reliés, 1,968 pp., 243 figures, 5 planches hors-texte en couleurs. Paris-VI^e^, France. Editions Médicales Flammarion, 22 rue de Vaugirard. Price (2 vols.) Fr. fr. 12,000.

Infektionskrankheiten, Vol. 1 of Handbuch der Inneren Medizin, 4th Edition, edited by G. v. Bergmann, W. Frey and H. Schwiegk; in 2 parts: part 1, pp. 1,536 with 417 illustrations, some in color; part 2, pp. 1,225 with 293 illustrations, some in color. Cloth. West Berlin, W. 35, Springer-Verlag (Reichpietschufer, 20), 1952. Price DM 374; by subscription, DM 299.

Third Report, Expert Committee on Health Statistics, including Second Report of the Subcommittee on the Registration of Cases of Cancer. World Health Organization, Technical Report Series No. 53. Geneva, W.H.O., Palais des Nations, 1952. Price 2/9; $0.35; Sw. fr. 1.40.

Fifth Report, Expert Committee on Biological Standardization. World Health Organization, Technical Report Series No. 56. Geneva, W.H.O., Palais des Nations, 1952. Price 1/3; $0.15; Sw. fr. 0.60.

BOOK REVIEWS

Industry and Tropical Health, proceedings of the First Industrial Tropical Health Conference, sponsored by The Harvard School of Public Health, December 8–10, 1950 at Boston, Mass. Boston: Published by the Harvard School of Public Health for the Industrial Council for Tropical Health, 1952. 316 pages, illustrated. Price: $10.00.

This beautifully bound book contains the full proceedings of a three-day Conference sponsored by The Harvard School of Public Health, attended by 37 delegates representing 23 industries, 9 of which are oil, 11 invited guests, and a good portion of the School faculty.

While the general theme of the Conference was "Industry and Tropical Health", the addresses and comments of the more than fifty participants, of necessity, at times went somewhat afield of the main theme.

The first four sessions, with corresponding chapters in the book, were organized around the major health problems peculiar to the Middle East, Tropical Africa, the Far East, and Tropical America respectively.

The remainder of the sessions were organized around topics of more general interest, and applicable on a more universal basis, though with particular reference to tropical areas. These include such matters as The Organization of Industrial Health Services, The Use of

Antibiotics in Certain Tropical Diseases, Nutrition, General Problems of Infectious Disease Control, and Various Aspects of Industrial Health Hazards. The real "meat" of the book, in this reviewer's judgment, is found in the latter part, dealing with these more generally applicable topics.

From the Public Health point of view, the most impressive and fundamental observation was made by Dr. Fred L. Soper in concluding his introductory remarks to Industry and Health in Tropical America. While recognizing that industry was faced with the more direct and immediate problem of maintaining isolated islands of people free from the diseases that afflict the populations surrounding them, "the problem as such," concluded Dr. Soper, "is insoluble." "A larger solution can come only with the development of satisfactory official local health services, official national health services and international organizations . . ."

Only as the surrounding populations are protected can health security for the industrially employed segment be fully achieved.

JAMES A. CRABTREE

Infective Hepatitis, British Medical Research Council Special Report Series No. 273. Studies in East Anglia during the period 1943–47, by F. O. MacCallum, A. M. McFarlan, J. A. R. Miles, M. R. Pollock and C. Wilson. 144 pp., paper. London: His Majesty's Stationery Office, 1951. Price 4/6.

This report serves, in part, the purpose of bringing together under one cover certain of the previously recorded accounts of the British experience with infective hepatitis both at home and abroad. Observations on the clinical course of disease, tests of hepatic function, hematologic alterations, and various experimental attempts to devise serologic tests as well as to transmit the disease to animals are well documented. In addition, the results of experiments in the transmission of hepatitis to volunteers are correlated with the observations made in other countries. Of new and special interest, however, is the section on epidemiology in which are discussed several aspects of the natural history of hepatitis as it occurred particularly among civilians from 1943–47 in a large and carefully studied area in England. The observations in this chapter supplement the information obtained from previous similar studies in Denmark and Sweden as well as earlier investigations in England.

W. PAUL HAVENS, JR.

Cardiolipin Antigens—Preparation and Chemical and Serological Control. World Health Organization Monograph Series No. 6, by Mary C. Pangborn, F. Maltaner, V. N. Tompkins, T. Beecher, W. R. Thompson and Mary Rose Flynn. 63 pages, illustrated. Geneva, World Health Organization, 1951. Price 5/-, $1.00, Sw. fr. 4.-.

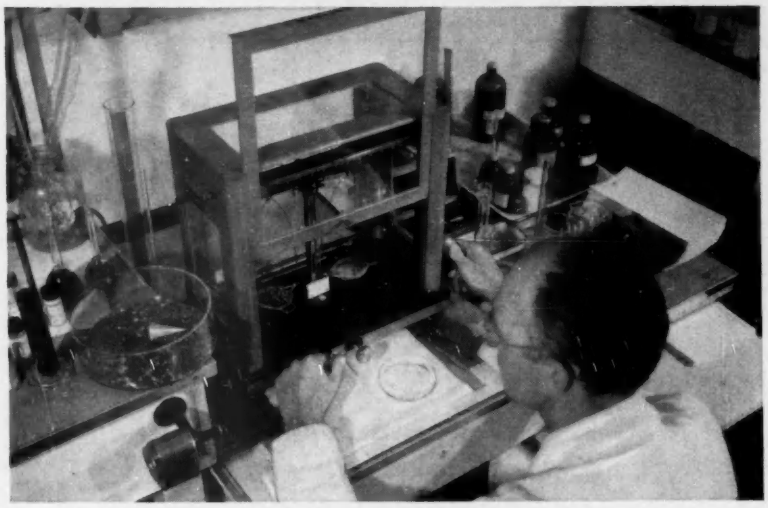
The present monograph has been prepared for WHO by the Division of Laboratories and Research of the New York State Department of Health. As indicated in the title, this volume presents detailed technical data for the preparation of the two phospholipids, cardiolipin and lecithin used in serologic tests for syphilis. Procedures for chemical and serological standardization are also included.

The problem of standardization of the reagents and methods used for the detection of Wassermann antibody has sorely beset workers in this field for many years. In this sense, therefore, application of the technics described in this monograph should mark notable progress. Unfortunately, however, there still exist several deterrents to international standardization of serologic tests for syphilis. Among these may be mentioned the technical difficulties involved in the actual preparation of cardiolipin and lecithin. These may be overcome in part through continued efforts directed towards the centralization of antigen manufacture and standardization in any given geographic area. Moreover, it would seem desirable at this time to consider a reduction in the number of flocculation technics all of which employ cardiolipin antigens yet differ amongst themselves in minor technical details.

A similar consideration applies to the complement fixation methods. The present monograph describes the modification of the latest technic originally developed by Wadsworth, Maltaner and Maltaner. While there is no question concerning the performance of this method, other possibly simpler technics with cardiolipin antigens are in current use. The ultimate choice of any method rests with the individual laboratory.

The monograph is well written and excellently organized. It would serve well as a constant reference manual for every laboratory engaged in the performance of serologic tests for syphilis.

A. G. Osler



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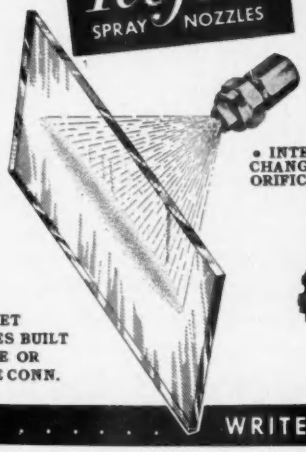

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APPLICATION FOR MEMBERSHIP

To the Society:

The following individual interested in tropical medicine and hygiene is proposed for active membership.

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Name .....
LAST FIRST MIDDLE

Mailing Address: City ..... State ..... County .....

Date of Birth ..... Place .....

Special Field of Interest .....

Education: Institution (Graduate and Postgraduate) Degree Date

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Attach to the application a list of professional positions held, membership in scientific societies, and a list of major publications.

*Proposed by:

(1) .....

(2) ..... Signature of Applicant

*Sponsorship by two active members is obligatory.

Return this application to Dr. Quentin M. Geiman, Secretary-Treasurer, Harvard School of Public Health, 25 Shattuck Street, Boston 15, Mass., U. S. A.

(*Check for $8.00 (U. S. banks requested) must accompany this application.*)

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and in arthropod borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted papers which raise no questions of scientific priority may however secure earlier publication if the author or the institution sponsoring the paper will pay the publication cost, an estimate of which the Editor will be glad to obtain from the publishers. Such a subvention will be used to purchase extra pages, in excess of the regular page allocation for any issue of the Journal.

Authors are asked to cooperate by complying with certain standard practices in the preparation of their manuscripts in order to facilitate the editorial task:

Papers should be written in *English*.

Manuscripts should be typewritten using *double or triple spacing* on *one side* only of 8½ x 11 inch paper, if available, and submitted in duplicate.

Tables should be typed on separate sheets and be serially numbered. Glossy prints of tabular material eliminate expensive typesetting.

Figures and *photographs* should be enclosed separately and protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be submitted on white paper or tracing cloth, and not on cross section paper. Colored illustrations can only be reproduced at the expense of the author.

References should be arranged in alphabetic order according to authorship, with the date following the author's name, e.g.:

1. Snyder, T., and Meleney, H. 1941, The excystation of *Endamoeba histolytica* in bacteriologically sterile media, *Am. J. Trop. Med.* **21:** 63–73.

Citation in the text should be by author and date.

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All papers should end with a brief summary.

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SMITH No. 44 GIANT DUSTER

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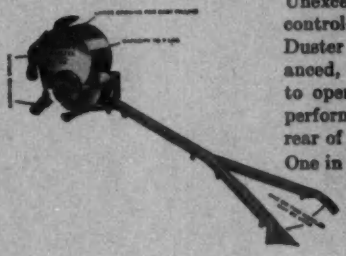


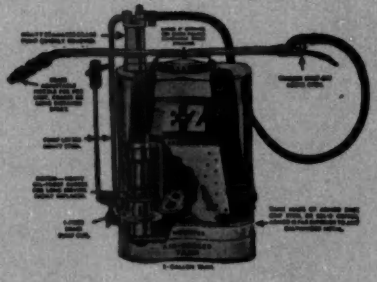
Unexcelled for mosquito and malaria control—when dusting is being done. Duster is precision built, well balanced, very light to carry and easy to operate. Superior in quality and performance. Also for dusting to the rear of operator.

One in case. Shipping weight—12 lbs.

SMITH E-Z KNAPSACK SPRAYER

Maintains continuous high pressure, with only easy slow pumping. Pump entirely of brass to stand long hard usage. Equipped with best grade, high-pressure hose, 2-foot brass extension pipe with adjustable brass or fixed type nozzle. New shut-off control may be locked open for continuous spray. Capacity 4 to 5 gallons. Has form-fitting ventilated tank, keeping the back dry and comfortable. Carrying handle; and dasher type agitator which keeps liquid thoroughly mixed. Brass liquid strainer.

One in case. Shipping weight—20 lbs.



D. B. SMITH & COMPANY • • UTICA 2, NEW YORK, U. S. A.

—— ESTABLISHED 1888 ——

DIFCO

DETECTION OF THE GONOCOCCUS

The superiority of the cultural method over the usual microscopic technique for detection of gonococcal infections has repeatedly been demonstrated. The cultural method is especially indicated in chronic and treated cases and for determining the release of patients. The use of the following standardized materials has simplified the diagnosis of gonorrhoea by the cultural method.

BACTO-G C MEDIUM BASE
enriched with Bacto-Hemoglobin and Bacto-Supplement A or Bacto-Supplement B is the most suitable medium available for cultural detection of *Neisseria gonorrhoeae* from all types of gonococcal infections. This new medium requires only 24 hours incubation. Upon this medium gonococcus colonies may be detected even when the organisms are present in the exudate in such small numbers that they may be missed upon microscopic examination.

BACTO-PROTEOSE NO. 3 AGAR
enriched with Bacto-Hemoglobin, is the original medium developed by Difco for isolation of the gonococcus and has been widely used for many years.

BACTO-SUPPLEMENT A
BACTO-SUPPLEMENT B
were developed as enrichments for chocolate agar prepared from Bacto-G C Medium Base or Bacto-Proteose No. 3 Agar with Bacto-Hemoglobin to furnish the essential growth factors required by some strains of *Neisseria gonorrhoeae*.

PROTEOSE PEPTONE NO. 3
is the basic ingredient of Bacto-G C Medium Base and Bacto-Proteose No. 3 Agar. This peptone, in a saline solution, is also recommended as a diluent for suspending the exudate prior to inoculation on chocolate agar.

BACTO-HEMOGLOBIN
in a two per cent solution is the preferred enrichment for preparation of chocolate agar from Bacto-G C Medium Base or Bacto-Proteose No. 3 Agar. It is readily soluble in water and is sterilized in the autoclave.

BACTO-DEXTROSE STARCH AGAR
is a very useful medium for propagation of pure cultures of the gonococcus. The organism grows luxuriantly upon this medium without further enrichment. Bacto-Dextrose Starch Agar also supports excellent growth of many other pathogenic bacteria and when used in half strength it is well suited for maintaining stock cultures.

BACTO-PHENOL RED MEDIA
are particularly useful for determination of fermentative reactions of newly isolated strains of the gonococcus. A selected group of complete agar and broth media are available, as are also the basic media without carbohydrate.

Leaflet No. 121 "Diagnosis of Gonococcal Infections by the Cultural Method" available on request

Specify "DIFCO"
THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
DETROIT 1, MICHIGAN